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UNIVERSITY OF ALBERTA

FAT ABSORPTION FOLLOWING SMALL BOWEL TRANSPLANTATION IN THE RAT

BY

Gerrit B. Winkelaar

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF **Master of Science**

IN

Experimental Surgery

DEPARTMENT OF SURGERY

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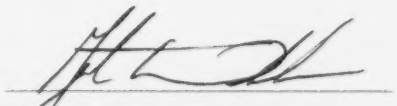

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Date: August 25, 1995

"To each one of us life is an experiment in Nature's laboratory and she tests and tries us in a thousand ways..."

- Sir William Osler, 1907.

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Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Fat Absorption Following Small Bowel Transplantation** submitted by **Gerrit B. Winkelaar** in partial fulfillment of the requirements for the degree of **Master of Science** in **Experimental Surgery.**

David L. Sigalet (Supervisor)

Lesley J. Smith (Co-supervisor)

Garth L. Warnock (Committee member)

Date: August 21, 1995

DEDICATION

To my parents, whose support has been unfailing,

to my wife, whose love, understanding, and patience have been boundless,

and,

to my children, who make it all worthwhile.

Abstract

Our goal was to study routes of fat absorption following small bowel transplantation (SBT) in the male Lewis rat. Control animals (n=19) underwent sham laparotomy. Experimental animals (n=15) underwent isogeneic orthotopic SBT with SMA-aortic and portal-infra-renal-IVC anastomoses. Both groups were followed for five to six weeks monitoring weight gain; balance studies quantifying absorption of dietary fat were performed in the last week. Animals then underwent cannulation of the mesenteric lymph duct, the portal vein draining the bowel, and the internal jugular vein. A duodenal tube was also inserted. Post-operatively, animals were restrained in Bollman cages. A lipid emulsion was infused through the D-tube until lymph flow stabilized. A bolus of radio-labelled medium and long chanin fatty acid was administered. Over the following 4 hours, blood and lymph was collected at regular intervals from each cannula. and animals then euthanized. All samples were analyzed for ^{3}H and ^{14}C labels in a scintillation counter. Groups were compared for recovery of fat from each cannula.

Dietary fat absorption in control animals was not significantly different from SBT animals. Lymph flow in control animals was significantly higher when compared to SBT animals. Mean total recovery of lauric and palmitic acids from the mesenteric duct of control animals was also significantly higher than SBT animals. On the other hand, mean fatty acid recovery from the jugular and portal veins of control animals was significantly lower than the corresponding values in SBT animals.

We concluded that, overall, absorption of dietary fat is maintained following SBT. However, lymphatic absorption via the mesenteric duct is significantly reduced after SBT. The significantly higher jugular fat recovery in SBT animals indicates that

after SBT. The significantly higher jugular fat recovery in SBT animals indicates that alternate pathways are functioning in fat absorption. Regenerative lymphatics in the retroperitoneum and around the arterial anastomosis were identified in selected animals using mesenteric methylene blue injection. These may be responsible for this alternate absorption. We also noted that lauric and palmitic acids were absorbed in similar proportions in the lymph despite their difference in chain length. These findings may have an impact upon dietary decision-making in patients who have undergone SBT.

ACKNOWLEDGEMENTS

I have never considered myself to be much of a basic scientist. That work which lies poised on the brink of discovery I always left up to the people I deemed to be much more brilliant and innovative in their thinking. I truly belived that cutting through the barrier of the unknown with the scalpel of experimental science required a certain outlook which I did not possess, and had no intention of obtaining.

However, this year has made me realize that experimental study is very much like the clinical surgery I enjoy so much: they both revolve around problem-solving, and, while frustrating at some points in time, both can give a sense of fulfillment and pride in one's accomplishments.

This about-face in my thinking is the result of working with a tremendous group of people, both personally and intellectually. Dr. David Sigalet is responsible for single-handedly pulling me from the depths of despair on numerous occasions when I thought the whole project was coming apart at the seams. His enthusiasm was so infectious that it gave me the ability to keep going even through trying times - I always left our meetings re-invigorated and eager to continue with the project. He also made me see that it is possible to balance a life of clinical and experimental surgery with a life at home - a challenge to which I hope I am equal. Finally, he taught me how to approach and solve problems in a basic science setting - a skill I intend to utilize and more fully develop in a clinical setting. For all these things, I would like to express to him my sincere gratitude and make him aware of what a great pleasure it was to work with him.

The other members of my advisory committe, Dr. Lesley Smith and Dr. Garth Warnock, I would like to thank for their insights into the project and their help in dealing with problems as they arose.

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LIST OF ABBREVIATIONS

Area Under the Curve	AUC
Cholecystokinin	CCK
Chylomicron	CM
Critical Micellar Concentration	CMC
Cyclosporin A	CsA
Decays per Minute	DPM
Diglyceride	DG
Epidermal Growth Factor	EGF
Fatty Acid	FA
Fatty Acid Binding Protein	FABP
Gastrointestinal	GI
Inferior Venal Cava	IVC
Long Chain Fatty Acid	LCFA
Medium Chain Fatty Acid	MCFA
Mesenteric Duct	MD
Monoglyceride	MG
Phosphatidylcholine	PC
Short Bowel Syndrome	SBS
Small Bowel Transplant	SBT
Triglyceride	TG
Very-low Density Lipoprotein	VLDL

Chapter I - Introduction

Fat absorption is a critical function of the small intestine. Fatty acids play a vital role in homeostasis through the provision of energy by their oxidation as well as their incorporation into cellular structural components. Although many fatty acids can be synthesized *in vivo*, there are several, such as linoleic and linolenic acids, which cannot and are therefore described as essential fatty acids. Since these fats must be ingested in order to be utilized, it is exceedingly important that fat absorption be efficient in order for the organism to avoid malnutrition.

In the ever-progressing age of organ transplantation and development of new immuno-modulation techniques, there has been a resurgence of interest in small-bowel transplantation (SBT)[81]. Although the indications for this procedure are still evolving, most of its clinical applicability revolves around the severe malabsorptive state known as the "short bowel syndrome". This syndrome has multiple etiologies, both congenital and acquired. Included in congenital causes are disorders such as midgut volvulus and apple peel anomaly of the superior mesenteric artery. Acquired causes include diseases which result in the removal of significant lengths of bowel such as necrotizing enterocolitis and Crohn's disease. The fundamental result of both congenital and acquired disorders is a significant loss of enteric absorptive surface area.

Numerous treatment modalities have been developed in order to combat this problem. Among these are intestinal lengthening procedures[51,84], reversed intestinal segments and creation of intestinal valves to slow transit of gut contents[200], and total parenteral nutrition (TPN). TPN is the most ubiquitous of the available treatment

options. However, it also has disadvantages including catheter sepsis[95], hepatic disease[30,129], and significant alterations in quality of life[118]. Small bowel transplantation was developed in order to allow for the re-establishment of the gut as the sole nutritional source, thus returning the patient to a nearly physiologic state without the need for TPN and its complications. However, the completeness of the return to enteic physiologic normalcy remains the focus of much study.

The procedure of SBT involves complete mesenteric lymphatic disruption which is not surgically corrected. The lymphatics, as will be discussed further, constitute the major route of fat absorption. The re-establishment of absorptive function for fat and fat-soluble vitamins is an important aspect of long-term survival and improved quality of life for SBT patients. If essential fatty acids are not absorbed well, these patients may still require intra-venous supplementation of these nutrients, thereby necessitating continued long-term intra-venous access - a problem which SBT is meant to obviate. Furthermore, the spontaneous development of a re-established lymphatic pathway without surgical anatomoses is important when considering potential post-operative problems such as lymphoceles and chylous ascites.

We are left with three basic questions regarding fat absorption following SBT. Firstly, do the lymphatics re-form, and if so, by what route? Secondly, does the bowel adapt in its fat absorptive function, perhaps by shifting from one absorptive route to another? Finally, is the overall fat absorptive function altered by SBT?

The asking of these questions prompted us to develop the hypothesis that fat absorptive function is significantly reduced following SBT. In these studies, we

attempted to test this hypothesis and thus develop an understanding of the physiologic alterations in fat absorption induced by SBT. In testing our hypothesis, our aim was to quantify the fat absorbed via the two main routes available to the bowel, namely the lymphatics and the portal circulation.

To begin, we will review the normal physiology of fat absorption and then discuss what is currently known about fat absorptive function following SBT. We will then turn our attention to a discussion of the accepted experimental models of SBT as well as the fat absorption models which provide the basis of our experimental work. An indepth description of our studies, results and the conclusions drawn from the data follows the literature review.

Chapter II - Review of the Literature

Fat Absorption Physiology

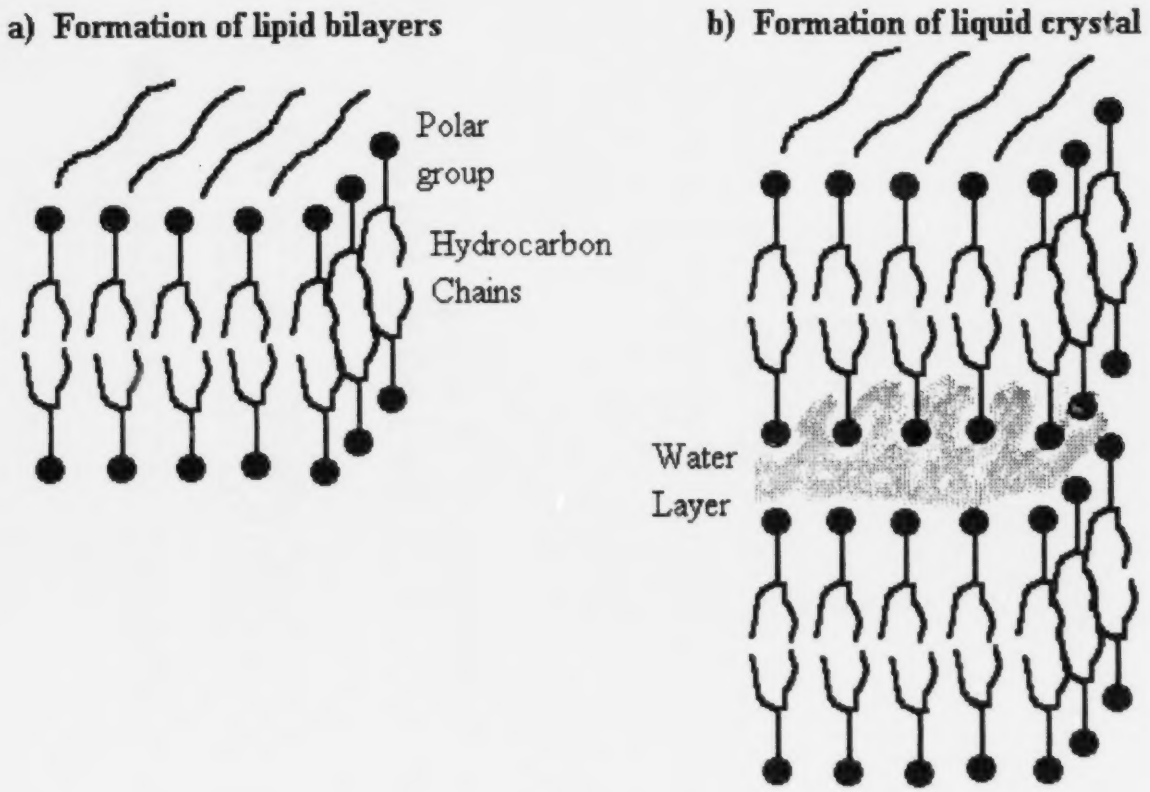
Fat absorption is a complicated process, many details of which are still under investigation.

To begin, a short review of fatty acids and their physical properties is required. The fundamental ability of a particular lipid moiety to interact with water dictates its bioavailability for intestinal absorption.[160] Shiau classifies polar lipids into three groups. Firstly, the insoluble non-swelling amphiphiles, of which triglycerides (TG), non-ionized long chain fatty acids and cholesterol are a part, have almost no interaction with water. They are notable in that they form a monolayer over the surface of an aqueous solution.

Lipids having a greater degree of polarity are able to interact with water beyond forming a simple monolayer. These lipids, known as insoluble swelling amphiphiles form liquid crystals. Liquid crystals develop in the following manner. First, lipid bi-layers form when individual lipid moieties coalesce in two lines with their hydrophobic ends together and their hydrophilic ends pointing externally as demonstrated in figure II-1(a). These bilayers then aggregate, sandwiching a layer of water between their polarized surfaces as seen in figure II-1(b). This "sandwich" of water between lipid bi-layers constitutes a liquid crystal. The formation of liquid crystals is termed "swelling". Lipids such as monoglycerides (MG), ionized fatty acids and phospholipids comprise this group.

Figure II-1

Creation of Liquid Crystals



The third group of lipids are the soluble amphiphiles. These are lipids with extremely strong polar groups which allow their dissolution in water at low concentrations. However, as the concentration of these lipids increases in an aqueous solution, they form micelles in which the polar groups are arranged outwards and the nonpolar ends of the molecules are turned inward creating a hydrophobic core. Bile salts are included in this group. However, unlike other lipids in this class, bile salts do not have the property of lyotropic mesomorphism. This behavior consists of lipids forming an intermediate liquid crystal phase as their concentration increases towards the critical micellar concentration (CMC). Instead, bile salts form micelles directly upon reaching the CMC. This allows them to more efficiently transport fatty acids (FA) in the lumen of the bowel.

Fatty acids (FA) themselves have interesting chemical properties. Saturated fatty acids have a basic structural formula of $CH_3(CH_2)_n COOH$. Unsaturated fatty acids have one or more double bonds between carbon atoms. Polyunsaturated FA have been receiving increased attention recently due to their role as precursors of prostaglandin and inflammatory mediators via the ω3 and ω6 pathways. Furthermore, the carbon double bonds are susceptible to peroxidation with production of oxygen free radicals. As these fatty acids are important components of many cell membrane phospholipids, their peroxidation can result in potentially serious cellular damage.[109]

Fatty acids are classified into three groups: short-chain $n < 6$, medium chain $6 < n < 12$ and long chain $n > 12$. Water solubility decreases with increasing chain length. Furthermore, the carboxylic group possessed by these molecules results in their physical

behavior being influenced by the environmental pH. At pH values lower than the FA pK_a, most long chain FA will exist as nonionized molecules and therefore will have little ability to interact with water. As the pH rises above the pK_a, the FA becomes ionized thus becoming an insoluble swelling amphiphile. This pH-dependent change which results in an improved ability to interact with water becomes important in the process of absorbing these molecules from the digestive tract.

Most fat in the diet consists of saturated fatty acids. The body is able to synthesize many saturated and unsaturated FA from these basic saturated FA. However, two polyunsaturated FA, namely linoleic and linolenic acids cannot be synthesized and are therefore regarded as essential fatty acids. Fat is ingested almost entirely as triglyceride.[188] TG has a glycerol backbone with three FA joined by ester bonds.

Triglyceride is an insoluble nonswelling amphiphile. Its inability to interact with water makes it nearly impossible for the intestine to absorb FA in this form.. The TG must be digested in order to provide lipid in a form with improved water interaction, thus making absorption easier.

Fat Digestion

Shiau[160] and Tso[187] divide digestion and absorption of fats into six stages: 1)intraluminal, 2)micellar solubilization, 3)absorption from lumen into enterocyte, 4)intracellular re-esterification, 5)chylomicron formation and 6)transport into circulation.

Intraluminal Stage

The intraluminal stage has two phases, the first being mechanical digestion. This begins in the mouth through the chewing of food creating small pieces of fat. When the fat particles reach the stomach, its powerful peristaltic action breaks down the particles and mixes them with the other ingested food and GI enzymes to created an emulsified chyme. At this stage, the lipid takes the form of 0.5 μm droplets which then enter the duodenum.[182] The net result of this process is to provide a higher surface area upon which the various digestive enzymes can act.

The second phase involves chemical digestion by GI - derived enzymes. Chemical digestion of fat actually begins in the stomach with the secretion of "pre-duodenal lipases". These lipases have been demonstrated to digest as much as 1/3 of the ingested fat[63,64] and are acid lipases with a pH optimum of 3.0 - 7.0.[62] Historically, controversy existed as to the source of this lipase. Some authors suggested that lipase activity in the stomach was derived almost exclusively from an oral source[12,182], citing its localization in the circumvallate papillae of the rat. On the other hand, others suggested that the origin of gastric lipase was not so clear.[100,188] More recent work, however, has demonstrated an unequivocal source of lipase in the stomach[72] through demonstration of its release in response to secretagogues. The cellular origin of this gastric lipase has been identified as the peptic cells located exclusively in the fundic mucosa, in association with pepsin.[115] It has also been demonstrated that in humans, the gastric lipase has by far the greatest activity of the pre-duodenal lipases.[29,114] Once

secreted, this lipase works exclusively on TG and has essentially no hydrolytic activity on phospholipids or cholesterol. It appears then that gastric lipase activity is the important first step in the chemical digestion of fat in humans.

Pancreatic lipase is secreted by the pancreas in response to food in the duodenum. The mural stretching as well as the chemical presence of partially digested proteins and FA's results in release of cholecystokinin (CCK) from the duodenal mucosa. CCK then stimulates the acinar cells of the exocrine pancreas to release their digestive enzymes. Gastrin, released primarily during the gastric phase of gastric secretion, also works with CCK to promote enzyme secretion from the pancreas. Secreted as an active enzyme, pancreatic lipase works optimally in a pH environment of 6 to 8. In the presence of bile salts, this optimal pH is reduced to about 6.0. Since gastric secretions entering the duodenum are about pH 1-2, significant alkalinization of the chyme must occur in order for lipase to work. The secretion of large quantities of bicarbonate by the pancreas is under hormonal control through the release of secretin from S-cells in the duodenum and upper jejunum. The primary instigator of secretin's release is intraluminal hydrochloric acid.[61] The bicarbonate secretion of the pancreas raises the pH of the gastric chyme to about 6.4 - well within in the optimal range of pancreatic lipase. Once lipase is released into the optimal alkaline environment, it has a complex interaction with its TG targets. In order to cleave the TG into free FA and 2-MG, lipase can only work at the interface between the aqueous chyme and the lipid droplet. More efficient TG digestion can occur if there is a greater surface area available upon which lipase can work, hence the importance of the mechanical digestion phase to reduce this rate-limiting step. Once at

the interface, lipase can be inactivated if it gets too close to the lipid surface due to hydrophobic bonding of the enzyme to TG. Somewhat paradoxically, bile salts, which function to improve FA absorption, can also inhibit pancreatic lipase when in concentration above CMC.[112,116] It is believed that bile salts inhibit lipolysis by inserting themselves into the oil-water interface, consequently displacing lipase from its active surface environment.[17] However, the presence of another protein, co-lipase, markedly reduces this inhibition.[18]

Co-lipase is a pro-enzyme secreted by the pancreas and broken down to its active form intralumenally by proteolytic enzymes. It then binds to the TG through the acyl ester bonds.[182] Lipase then tightly binds to the co-lipase protein through electrostatic interactions. The lipase is thus kept in proper proximity to its target molecules. The importance of co-lipase's activity in maintaining the relative position of lipase cannot be over-emphasized. Without the proper positioning of the lipase enzyme provided by co-lipase, TG hydrolysis would be an inefficient process, most likely resulting in significant fat malabsorption. It is also theorized that co-lipase displaces bile salts from the oil-water interface, thus allowing for the re-establishment of lipase's optimal position adjacent to the interface.[19] Thus it is that the normal intestinal digestion of fat depends on the presence of co-lipase in order to ensure the proper function of pancreatic lipase in an environment to which it is extremely sensitive.

Interestingly, gastric lipase does not appear to have a susceptibility to inhibition by bile salts and does not require a co-lipase factor.[62] Indeed, gastric lipase is of prime importance in fat digestion in newborns, as the pancreatic lipase/co-lipase system is not

yet fully developed.[188] Furthermore, it appears that ingested milk fat forms globules which are resistant to the activity of pancreatic lipase but not gastric lipase.[24] It has also been demonstrated that immersion of these ingested globules in a gastric lipase "bath" also improves the subsequent action of pancreatic lipase upon the milk triglycerides. According to Hamosh, up to 70% of dietary fat can be digested and absorbed in the absence of pancreatic lipase through the activity of gastric lipase.[63]

The gastric lipase system is also important later in life in conditions such as cystic fibrosis and other disorders causing pancreatic insufficiency. In these cases, where pancreatic lipase is not delivered to the intestinal lumen in sufficient quantities, it appears that gastric lipase activity increases appreciably in order to compensate for an otherwise malabsorptive state.[62] Currently, the treatment of these pancreatic insufficiencies involves the oral administration of pancreatic enzyme supplements. Unfortunately, less than 8% of the lipase given through these supplements arrives intact at the ligament of Trietz.[35,111] This loss is most likely due to denaturation and inactivation in the low pH environment of the stomach. Thus, it has been suggested that lipase supplementation in these conditions should take the form of gastric lipases which would maintain their function with oral administration.[62] As human gastric lipase has been cloned, this approach holds promise for the future for patients suffering from these diseases.

Micellar Solubilization

To understand this step in fat absorption, a review of the physiology of bile salts is necessary. Bile salts are produced by the liver and are secreted into the biliary tree to enter the intestinal lumen. Primary bile salts, cholic and chenodeoxycholic acids, are produced by the hepatocytes in quantities of about 0.5 g each day. These salts are then conjugated with either glycine primarily or taurine to a lesser degree prior to their secretion. Bile salts are highly conserved by the body. After entrance into the intestine and functioning to promote fat absorption, they are reabsorbed through both passive and active mechanisms in the bowel. Experiments using an intestinal intubation technique demonstrated a reduction in lumenal dihydroxy bile acid concentrations as the bile salts moved down the small intestine.[5] Active transporters of bile acids in the upper small bowel have not been identified.[2,16,166] Furthermore, it has been demonstrated that the amount of bile salts absorbed through the acitve mechanisms of the human ileum are not sufficient to account for the total bile acid absorption by the small intestine.[151] This evidence suggests that glycine-conjugated bile acids are absorbed passively in the upper small intestine to at least a limited extent and that the active transport mechanisms isolated to the terminal ileum probably serve to "mop up" those bile salts which are not absorbed higher in the enteric stream. Passive absorption probably occurs through protonation of the bile salts as they approach the acidic unstirred water layer adjacent to the enterocytic brush border. They then "flip-flop" across the lipid-rich enterocyte membrane into the cytosol.[71]

It is estimated that about 15 g of bile salts are required for fat absorption from each meal.[61] With a gallbladder bile salt pool of about 5 g, this requires three circulations per meal. Thus, the active absorption of the terminal ileum is a very important safety net in the conservation of bile salts; without it, the loss of bile salts could be sufficient to cause a fat malabsorptive state[71] before hepatic synthesis could compensate for the loss. It is conceivable that this effect may be responsible for the fat malabsorption demonstrated in patients who have undergone terminal ileal resections or who have terminal ileitis. The active process of bile acids appears to be carried out by a Na^+/anion co-transporter. This transport system utilizes a sodium gradient created by the export of sodium ions from the enterocyte by baso-lateral membrane Na^+/K^+ ATPases to transfer the bile acid into the enterocyte.[71] The regulation of this transport system thought to be essentially non-existent, operating under conditions of saturation in healthy individuals. More recently, experiments in bile-acid feeding have demonstrated down-regulation of active transport proteins, suggesting a kind of feedback inhibition.[102] These interesting results require further confirmation, but do suggest that some form of regulation of this process is operating.

Once in the enterocyte, the bile salts are bound by a bile-acid binding protein[203], presumably to prevent their harming intracellular organelles.[94] The bile salts are then transported out of the enterocyte in exchange for other anions such as bicarbonate by a specific transport protein in the basolateral membrane.[203]

Those bile acids which escape reabsorption are metabolized to secondary bile salts, deoxycholic and lithocolic acids, and also de-conjugated by colonic flora.

Deoxycholic acid is reabsorbed passively in the colon[71], whereas lithocholic acid, being highly insoluble, is lost in the feces. The passive reabsorption of deconjugated bile salts probably involves the induction of secretion of chloride ions from the enterocyte.[33] It is reasonable to suggest that if excessive amounts of bile acids reach the colon, a secretory diarrhea may result through the over-stimulation of this passive re-absorptive pathway. Thus, it appears that the terminal ileal active absorption of bile acids also constitutes an important barrier to the passage of bile salts into the colonic lumen.

Once absorbed into the enterocyte and then released, the bile salts are taken by the portal blood to the liver where they are taken up by the liver to be re-conjugated if necessary and re-secreted into the bile. This re-cycling of bile salts is termed the enterohepatic circulation and its proper function is necessary for the maintenance of normal fat absorption.

The bile salts have two functions in fat absorption. Firstly, they act as a detergent to break up the fat particles even more than in mechanical digestion. thus improving the surface area for lipase to act upon even further. In addition to this emulsifying function, they form micellar aggregates with FA and MG to facilitate their absorption. The first step in this micellar process is to get enough bile salts in solution to reach the critical micellar concentration (CMC). This concentration varies widely depending on the particular bile salt, the temperature, pH, presence of other lipids and the ionic composition of the solution.[160] As mentioned previously, the micelles exist as a sphere with the polar ends outwards and the hydrophobic ends turned inwards into a central core. The products of lipolysis are removed from the oil-water interface by incorporation

into the hydrophobic cores of the micelles as they mix in the chyme. These fatty acids and 2-monoglycerides are almost entirely ionized due to the more alkaline pH of the duodenum. They are therefore insoluble swelling amphiphiles which easily dissolve in micelles. Thus, the process of micellar solubilization simply improves the movement of these water-insoluble products through the aqueous medium of the chyme to the mucosal surface. In fact, incorporation of 2-MG into micelles enhances the dissolution properties of the micelles so that they are able to solubilize more nonpolar compounds such as cholesterol, thus improving their absorption as well. This behavior is known as "enhanced solubilization" or "co-operative effect".[72,183]

Once the micelles have delivered their digestive products to the mucosal surface, it appears that they have two potential fates. Firstly, they may diffuse back through the luminal contents to collect further fat digestive products for absorption. The second potential outcome is that they may lose some of their bile salts through passive absorption by the enterocyte as they are protonated in the unstirred water layer next to the mucosal surface.[71] The gradual reduction in lumenal bile salt concentration induced by their reabsorption with passage down the gastrointestinal lumen would result in less efficient fat absorption in the lower small intestine and thus provides additional evidence that most of the fat absorption probably occurs in the upper small intestine as suggested by Simmonds.[166]

Micellar solublization encourages a much greater efficiency in fat absorption by the gut by increasing the aqueous concentration of FA and monoglyceride (MG) 100 to 1000 times.[187] However, the formation of these micelles in sufficient concentration to

ensure proper fat absorption depends upon the integration of many processes. Among these are hepatic synthesis of bile salts, passive and active bowel absorption of bile salts and the enterohepatic circulation, any of which may become disrupted through a number of disease processes, resulting in a fat malabsorptive state.

Enterocytic Absorption (Figure II-2)

Simmonds et al. in 1967 [166] demonstrated that the absorption of fats from a test meal was almost entirely completed before the meal reaches the middle third of the jejunum. The complexities of this absorption have been the subject of much investigation.

Shiau [160] identifies two barriers to FA absorption in the gut. The first of these is an aqueous barrier next to the mucosa generally referred to as the "unstirred water layer". Permeation of this layer by these insoluble lipids is afforded by the micellar solubilization described above. Due to the excellent aqueous dissolution properties of micelles, they are able to carry the FA and MG through the aqueous luminal "bulk" phase toward the mucosa. They can also penetrate the aqueous barrier with their passenger lipolytic products, thus delivering the FA and MG to the enterocyte membrane in high concentrations. Permeation of this unstirred layer has been identified as the overall rate-limiting step in long chain FA and cholesterol absorption.[134,204] It is well-known that lipolytic products are absorbed as monomers and not as micellar aggregates.[Tso,182,188,206] Thus, FA must somehow free themselves from the micelles. An "acidic microclimate" identified by Shiau and Levine[159] and independently by Daniel[26] is encountered by the

micelles on the other side of the unstirred layer immediately adjacent to the enterocyte membrane. The high proton concentrations in this microclimate are attributed to the presence of a mucus coating and not to active proton pumping.[182] The FA and MG are believed to dissociate from the micelles under the influence of the reduced pH in this environment. This is likely due to protonation of the polarized end of the FA and consequent loss of its swelling property which no longer allows its micellar solubilization. However, this dissociation hypothesis is controversial and requires experimental confirmation. If the dissociation hypothesis is proven correct, it is most likely that most of the FA absorption occurs at the villus tips due to the presence of the acidic microclimate in these areas with rapid reduction in proton levels as one descends into the crypts.[26]

Once dissociated from the micelle, the lipolytic products encounter the second barrier to absorption - the enterocyte lipid membrane. The primary lipid components of this membrane are phospholipids and cholesterol.[43] The important factor in penetration of this barrier is the permeability coefficient of the fatty acid to be absorbed. This coefficient increases with chain length.[160] Longer chain FA, once protonated in the acidic microclimate are highly lipid soluble and therefore cross the membrane more rapidly through a process of simple passive diffusion.[144] With FA up to 16 carbons long, permeation into the enterocyte likely involves intercalation within the hydrophobic core of the cellular membrane[132,133] and subsequent passage into the cytosol. The barrier presented by the cellular membrane appears to constitute the rate-limiting step of

MCFA[160] absorption, probably due to the relatively decreased lipid solubility of these molecules.

Evidence for a carrier-mediated FA absorption process has been demonstrated more recently. Stremmel, in 1988, identified a fatty acid binding protein associated with the enterocyte membrane.[173] His work suggested that this protein may play a significant role in the enterocytic absorption of LCFA as well as phosphatidylcholine and cholesterol. Confirmation of a carrier-mediated process of FA absorption was provided earlier by Chow and Hollander.[22] In addition, they were able to demonstrate that the function of this carrier protein appeared to be dependent upon lumenal FA concentration. Briefly, they were able to show that with low concentrations of linoleate in the bowel lumen, carrier-mediated absorption predominated. However, with higher linoleate concentrations, absorption occurred primarily through passive diffusion.

In general, then, passive diffusion appears to be an important process in the absorption of FA from the bowel lumen into the enterocyte. The newer work demonstrating an active transport mechanism in this process is exciting. However, more investigation is required in order to more precisely define the role this pathway plays.

Intracellular Re-esterification

Once absorbed, the intra-cellular events surrounding the metabolism of FA and MG are only partially understood. The goal of these events is to prepare the lipolytic products for transfer into the systemic circulation. This primarily entails re-esterifying the FA and MG back into TG.

Since these products remain insoluble, they are not freely dispersed in the cytosol.[160] An intra-cellular fatty acid binding protein (FABP) identified by Ockner and Manning[120] is believed to be involved in transporting FA and MG to the smooth endoplasmic reticulum (s-ER) for re-esterification. This protein has been demonstrated in greater quantities in the jejunum versus the ileum, a finding which helps explain the preferential fat absorption properties of the upper intestine as suggested by Simmonds.[166] In addition, Ockner and Manning were able to demonstrate that this protein occurred in greater concentrations in the villi than in the crypts and in the enteric mucosa of animals fed a high fat diet rather than a low fat diet. Thus, it appears that this protein must be important in the absorptive function of fat.

It has been demonstrated that this FABP has a higher affinity for LCFA than MCFA[120], although the chain length threshold for this affinity has not yet been established. The basis for this difference in affinity may lie with the shape of the binding site. Using high resolution x-ray crystallography, Sacchettini et al.[143] have demonstrated that the FABP isolated from rat intestine has the appearance of a clam with the binding site inside the protein itself. Binding of FA appears to occur through a complex hydrogen bonding with the carboxylate group of the fatty acid. Once bound, the FA hydrocarbon chain undergoes a slight conformational change due to surrounding hydrophobic and aromatic amino acids, taking on a slight helical twist in order to lie properly within the binding site. In the binding of any FA, it is likely that the FA enters the binding protein through a channel and forms the carboxylate-hydrogen bonds as a sort of anchor. However, with shorter chain lengths and the inherent solubility changes this brings, it is

possible that the hydrocarbon chain conformational changes either cannot occur, due to altered alignment of the chain with the bed of the binding site, or they are insufficient to allow proper seating of the FA in the binding site. Thus, shorter chain FA would not be bound by FA to the same degree as LCFA.

It also appears that a single FABP carries only one FA molecule at a time.[143] However, once bound, the FA can be moved through the enterocyte's cytoplasm, thereby ensuring its proper metabolism within the cell.

Ockner and Manning have hypothesized that FABP binding is critical for re-esterification.[120] That is, FABP acts as a shuttle for absorbed FA to reach the s-ER and subsequently undergo re-esterification to TG. As such, those FA which have good affinity for FABP are channeled into a re-esterification pathway with subsequent chylomicron formation as we shall see. On the other hand, those FA having a lower affinity for FABP reach the s-ER for re-esterification in much smaller amounts, and may pass directly into the portal vein in their un-esterified form. This provides an explanation for the observations of Bloom, Chaikoff and Reinhardt who demonstrated in 1951 that LCFA was absorbed primarily through the lymphatic system and that MCFA was absorbed in much lower quantities via this route.[13] They surmised that MCFA must then be absorbed primarily by the portal blood. However, they did not directly measure the portal vein absorption and thus could not objectively reinforce this inference.

In summary, the relative affinity of a particular FA for FABP may be the most important factor in regulating its metabolic fate within the enterocyte and its subsequent transfer into the systemic circulation. Thus, FABP may act as a "gate" of sorts in FA

absorption, and may be responsible for the observed differences in absorptive routes taken by FA based on their chain length.

Re-esterification occurs primarily by the monoacylglycerol pathway[188] through the action of an enzyme complex called "triglyceride synthetase".[79] This pathway utilizes absorbed 2-monoglyceride as a skeleton upon which to attach FA in the 1- and 3-positions. It is believed that this pathway occurs primarily on the cytoplasmic surface of the smooth ER.[7] A second available re-esterification pathway involves the stepwise acylation of glycerol-3-phosphate to TG. Referred to as the α-glycerophosphate pathway, it becomes important only when the amount of 2-MG is insufficient to support the monoacylglycerol pathway.[188]

Once formed, the TG then gains entrance to the ER lumen. It appears that a microsomal TG transfer protein in the ER membrane may be responsible for this shuttling of surface-formed TG into the ER for further packaging.[187] It has been suggested by Wetterau et al.[205] that patients with abetalipoproteinemia lack this TG transfer protein, thus creating an intracellular block in TG metabolism leading to the characteristic build up of fat in the enterocytes of these patients.

Once re-esterification has occurred, the TG undergoes further packaging into chylomicrons and other lipoproteins prior to its transfer into the systemic circulation.

Chylomicron (CM) formation

Chylomicrons are the major lipoprotein carrier of TG during feeding.[160] Very low-density lipoprotein (VLDL) is the major lipoprotein in the lymph during fasting. Tso

et al. have hypothesized that separate pathways are responsible for producing each of these lipoproteins, possibly based on the re-esterification pathway utilized.[190] TG produced by the monoacylglycerol pathway appears to stimulate chylomicron formation. On the other hand, TG synthesized by the α-glycerophosphate pathway results in VLDL formation. They demonstrated this differential packaging by using a hydrophobic surfactant known as Pluronic L-81.

From their studies, it appears that Pluronic L-81 impairs the packaging of TG into CM via the monoacylglycerol pathway. However, the packaging in VLDL of substances which are metabolized only through the α–glycerophosphate pathway, such as phosphatidylcholine, remains unaffected by Pluronic L-81. Thus, it appears that separate metabolic pathways are responsible for producing CM and VLDL and that the metabolic route of re-esterification taken by an absorbed fatty acid will predict its eventual packaging fate.

The metabolic route of re-esterification appears to be based on the availability of pre-cursors.[190] Briefly, the monoacylglycerol pathway requires the presence of 2-MG upon which to sequentially attach FA molecules. Without 2-MG, the α-glycerophosphate pathway predominates.[187] This precursor hypothesis also helps to explain the differences seen in CM and VLDL levels under fasting and starving conditions: during feeding, the amount of 2-MG is sufficient to drive the monoacylglycerol pathway and thus, CM are formed in greater quantity. Conversely, during fasting, there is very little 2-MG available, thus the α-glycerophosphate pathway takes over the re-esterification function of the enterocyte, resulting in greater VLDL production.

Chylomicrons appear to be created in the following manner. Once the re-esterified TG penetrate the ER membrane, they aggregate and become associated with phospholipids and apo-proteins in "pre-chylomicrons" through a mechanism which is still under investigation.[187] The origin of the phospholipids is thought to be phosphatidylcholine (PC) absorbed from the bile as lyso-PC which is then re-esterified to PC intra-cellularly.[106] *De novo* synthesis of PC does not appear to be important in chylomicron formation. It appears that the luminal supply of PC is important in the formation of CM, although the reason for this is only speculative.[187] PC is important in creating a normal CM coat. The need for a biliary source of this PC is not clear but may be due to an enhanced handling of biliary PC by the enterocyte based on the its unique FA composition. The apo-proteins, including apo- A-I, A-IV and B-48 which are the primary CM proteins, have been demonstrated in the rough ER, their likely site of origin. As well, the Golgi apparatus has been demonstrated as the site of stored chylomicron sugars[82] which would indicate its role in glycosylation of the pre-CM particles. Since the smooth and rough ER as well as the Golgi are all a part of the endomembrane system[117], it is likely that chylomicron formation does not occur in a single organelle.[82] They may be transferred from one organelle to another either within this membrane system or by a process of budding off from the smooth ER and merging of these "buds" with the Golgi apparatus. Another "shuttle" system consisting of microtubules connecting the s-ER to the Golgi, called the "boulevard périphérique" has also been suggested.[117] The importance of a micro-tubular system has been emphasized by demonstrations of significant reductions in the appearance of CM in the lymph of rats

who had been administered known the microtubular poisons cholchicine and vincristine.[187]

The Golgi apparatus is important in preparation of pre-chylomicrons. The pre - CM phospholipid composition is increased and the protein content is reduced in the Golgi.[187] In addition, the Golgi serves as the site of terminal glycosylation of the pre-CM prior to its release into the systemic circulation.[182]

The end result of this enterocytic procedure of formation processing of absorbed FA is a spherical "package" 0.03 - 0.5 μm in diameter made up of triglyceride and varying amounts of cholesterol in its centre.[61] The size of the CM formed appears to be influenced by the amount of fat absorbed.[569] Arrayed around this fatty "core" are phospholipids with their hydrophobic ends directed inward and their charged ends pointing outward. This external charge allows for the free diffusion of CM in the aqueous fluids of the lymph and vascular systems. The structural similarity between CM and luminal micelles is no accident; without the amphipathic properties of bile salts and phospholipids, absorption of insoluble fats into the aqueous environment of the body would be seriously impaired.

Arrayed externally with the phospholipids to complete the "shell" are the apo-proteins. These packages are then extruded from the Golgi apparatus in preparation for their transfer into the enteric lymphatics.

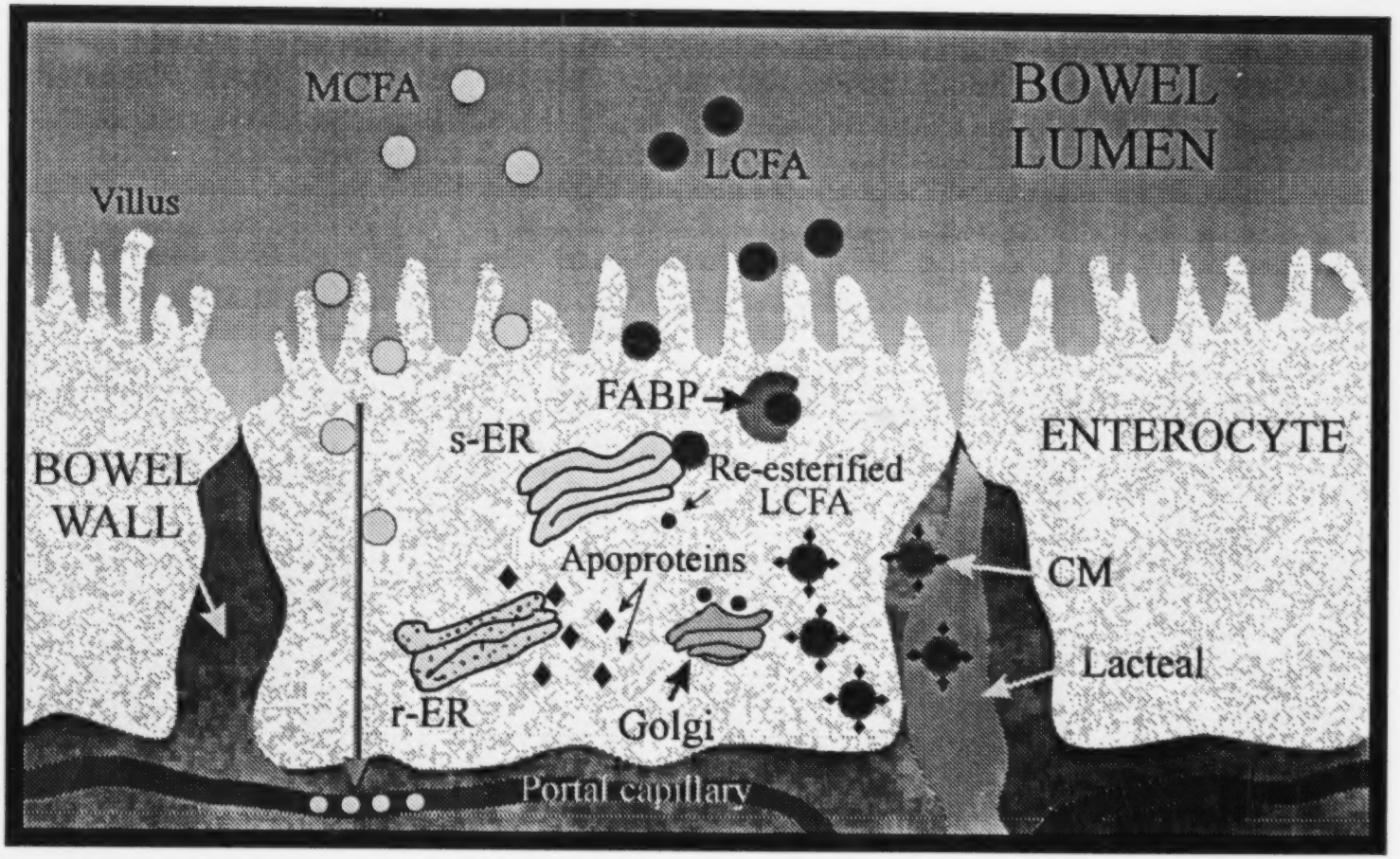
Transfer into Systemic Circulation

Golgi-derived vesicles appear to contain either CM or VLDL; very little mixing of the two seems to occur.[187] These vesicles move to the lateral membrane of the enterocyte and merge with it. The apo-B moieties on the outer surface of the CM may play an important role in attachment of the particles to the lateral membrane.[61] The CM and VLDL are then discharged into the intracellular space through a process of membrane diffusion. This process appears to be enhanced by the presence of ionized calcium.[187] The fate of the Golgi vesicle membranes is not clear; they are not exocytosed with the CM and CM do not contain surface membranes. Thus, although controversial, it has been hypothesized that the Golgi vesicle membrane simply becomes a part of the enterocyte cellular membrane and enters a membrane recycling process.[187]

Once discharged, the CM then enter the lacteals of the villi which coalesce into mesenteric lymphatics. These lymphatics then drain into the mesenteric duct which then flows into the cisterna chyli. Here, the thoracic duct arises and the fat-laden lymph is transported through it and into the venous system.

In summary, the digestion and absorption of fats requires an integration of numerous para-enteric organs and their secretions as well as a properly functioning bowel mucosa and drainage pathways, including lymphatics. Furthermore, the role of neural and hormonal control is still under investigation.[187] It is obvious, then, that absorptive function of fats can be disrupted at any number of levels.

Figure II-2
Fat Absorption Physiology



Alterations in Absorption Physiology

The exceedingly large absorptive surface area of the small intestine is necessary in order to derive maximal nutrition from ingested food. However, many disease processes alter the ability of the gut to adequately perform this function. Central to our discussion are the changes wrought by massive bowel resection and by small bowel transplantation.

Massive resection

The result of massive small intestinal resection is a global malabsorptive state called the "short bowel syndrome"(SBS). With loss of a significant length of absorptive surface, the bowel's absorptive capacity is overwhelmed resulting in intractable diarrhea, steatorrhea, malnutrition, and weight loss. The severity of this absorptive dysfunction depends upon many factors. These include length of resection and coinciding surface area loss, proximal or distal resection site, preservation of the ileo-cecal valve, and the baseline and adaptive ability of the residual small intestine.

There appears to be clinical agreement that resection of up to 75% of the small bowel can be tolerated.[39,184,193] Animal studies by Reynell and Spray[138] demonstrated that resections of up to two-thirds of the small bowel were compatible with normal absorptive function.

The site of resection is important as there are specific functions of identifiable areas of the small intestine which cannot be replaced. For example, the duodenum and upper jejunum are important sources of enteric hormones. Loss of these areas results in

reduced CCK and secretin release. As a result, pancreatic and biliary secretions are reduced.[39] Furthermore, hypergastrinemia results from a decreased secretion of gastric inhibitory polypeptide and vasoactive intestinal polypeptide[172] due to jejunal loss. Consequently, gastric secretion continues unchecked. The high fluid load of the chyme entering the upper residual small intestine causes diarrhea in itself. In addition, the extremely low pH of the chyme inactivates digestive enzymes, thus compounding the malabsorptive state. The terminal ileum has specific functions which cannot be replaced by adaptive bowel changes. Specific active transport pathways mediate bile salt absorption[21,34,70,96] as discussed previously. Another important function is the absorption of vitamin B_{12} in the terminal ileum. Loss of this particular nutrient into the large bowel results in enhanced bacterial overgrowth due to its utilization by colonic flora. Finally, a competent ileo-cecal valve is necessary in controlling intestinal transit time[39] and preventing reflux of bacteria into the small bowel from the colon, hence preventing bacterial overgrowth. Preservation of this regulatory gate can double the absorptive function of the residual bowel by reducing transit time and therefore keeping the chyme in contact with the bowel surface for a much longer period.[39]

In addition, the presence of fatty acids in the terminal ileum has been shown to induce the release of entero-glucagon. The release of this hormone results in a slowing of intestinal transit as well, a feedback loop termed the "ileal brake".[169] This process is an important regulatory mechanism which plays a role in both proximal and distal resections. Firstly, following proximal resections, the remaining proximal intestine may be overwhelmed by the nutrient load presented to it. As a result, unabsorbed FA will

reach the terminal ileum and stimulate the ileal brake, thus increasing the mucosa-chyme exposure time in order to maximize proximal absorption. Conversely, with distal resections, the originating site of this feedback loop may be lost. Again, with a smaller absorptive surface area following resection, the proximal bowel may be unable to achieve maximal absorption. However, with the inability to induce the ileal brake, the malabsorptive state goes unchecked. Thus, the importance of this mechanism in regulating nutrient absorption cannot be over-emphasized. This also may explain why distal resections, resulting in a loss of this feedback loop, are not as well tolerated as proximal resections as will be discussed next.

The literature is filled with studies which have shown that distal resection is less well tolerated than proximal resection. Reynell and Spray[138] demonstrated a higher weight loss and greater defects in glucose and iodide absorption in animals undergoing distal versus proximal resections. They also showed a more rapid transit time in association with distal resections. It may be that this rapid transit time is due to loss of the ileo-cecal valve, or as mentioned previously, the ileal brake. It may also have its origin in reduced neuromuscular activity of the bowel incurred by massive resection. The loss of weight in distal resection animals could be overcome by a greater food intake, as shown by Young and Weser.[210] Thus, presenting more nutrients to the shortened bowel surface seems to overcome the effect of rapid transit time on reduced absorptive function. Eighty percent distal resection has also been demonstrated to increase jejunal as well as colonic permeability.[196] This may indicate that absorptive

dysfunction of the proximal remnant on a cellular level may also be induced by massive distal resections.

Proximal resections appear to be better tolerated due to an extraordinary ability of the distal bowel to adapt and thus increase its absorptive capacity. The mechanisms surrounding this adaptation process have been studied extensively. The feature noted most regularly in the residual bowel is villous hypertrophy and dilation.[37,38,66,67,86,206] The phenomenon of residual bowel elongation has not been consistently demonstrated. The hypertrophy takes the form of increased villous height and crypt depth. Increased mucosal mass has been confirmed by elevated DNA, RNA and protein.[66,206] As the baseline villous height is much less in the ileum[25], this adaptive response is much greater in the distal small bowel and is proportional to the length of bowel resected.[66] While unable to confirm villous hypertrophy, Nylander and Olerud[119] did find that post-anastomotic dilation occurred, usually involving about one-third of the remaining bowel, gradually tapering to normal ileal diameter. They suggested, that, due to this dilation over a prolonged segment of bowel, transit time was decreased, allowing more time for chyme-mucosal contact and, therefore, nutrient absorption. Since the unhypertrophied villi had normal capillary structure without arterio-venous shunting, they would have full absorptive capabilities. Thus, dilation alone, they surmised, provided the bowel with its adaptation. Furthermore, they suggested that, since proximal resections tend to leave a longer length of bowel which is able to undergo adaptive compensation, these resections would tend to be better tolerated.

The impetus for the villous hypertrophic changes has not been entirely elucidated. Of prime importance seems to be luminal nutrition. Delivery of a much higher nutrient load to the residual distal segment may induce this adaptive change[36,196] Experiments in which jejunal loops were transposed for ileal loops revealed some good evidence for this theory.[38] The ileal loops, once placed upstream in the enteric flow, showed marked hyperplastic changes. The jejunum, being downstream, received less nutrition and underwent atrophy. Furthermore, when jejunal bypass was performed, the bypassed segment also showed marked atrophic changes.[53] However, being entirely out of the enteric stream, these loops were also not exposed to pancreatico-biliary secretions which may have trophic capabilities as well. This theory was initially put forward in 1971, through the experiments of Altmann and Leblond[3,4] who diverted the pancreatico-biliary stream into loops of ileum and demonstrated marked hypertrophy in these loops.

Hormonal sources of this hypertrophy have also been studied. This theory was developed in response to observations that the atrophy expected in bypassed loops could be reduced or even reversed by performing a simultaneous resection of bowel in the enteric stream.[36] In these cases, it would appear that the second resection may initiate the release of trophic hormones as a part of the healing response. Thus, specific hormones have also been investigated.

Although initially implicated, gastrin appears to not play a role in adaptive hyperplasia.[31] CCK has also not been shown to induce these changes.[36] A number of other hormones, including anterior pituitary hormones and the hormonal milieu of lactation, have received interest but no firm conclusions as to their effect on adaptation

have been developed. Sensory neuropeptides have also been shown to have no effect on the regulation of intestinal cell growth.[11]

One trophic hormone which has been shown to play a role in bowel hyperplasia is epidermal growth factor (EGF). Interest in this hormone resulted from the observation that breast milk incited neonatal intestinal growth and contained significant quantities of EGF.[9] Studies have shown that EGF increases the brush-border surface area, probably from intracellular stores, within 2 hours of EGF administration.[68] With more chronic administration, increased intestinal mucosal DNA and intestinal weight and length result.[9] It appears then, that EGF has an important role in inducing hypertrophic changes in the intestine. However, Goodlad et al. showed that resection itself did not induce an increased sensitivity of the residual bowel mucosa to EGF: no synergy between administered EGF and resection on intestinal hypertrophy was demonstrable.[54] While EGF was still effective in inducing intestinal hypertrophy, EGF in addition to luminal nutrition provided a greater response following resection. In addition, resection was shown to increase the level of circulating enteroglucagon, indicating a possible role in adaptation played by this hormone. However, EGF-associated proliferation was not associated with elevated enteroglucagon, suggesting that the two hormones work by separate mechanisms. From this work, it appears that the hormones EGF and enteroglucagon probably play at least some role in the adaptive response following intestinal resection. Further study in this area is warranted. Specifically, investigations into the effect of resection on circulating levels of EGF may demonstrate a more precise pathway by which post-resection intestinal adaptation in achieved through

hormonal release. In addition, the elevated level of enteroglucagon and its implied role in the adaptive response by Goodlad et al.[54] is an interesting finding in the light of the "ileal brake" put forward by Spiller et al.[169] As described earlier, it appears that the presence of FA in the ileum stimulates the release of enteroglucagon which results in slowing of small bowel motility. Following intestinal resection, the initial malabsorption of fat would result in just such a situation. Thus, it is logical to suggest that the effect of enteroglucagon post-resection is two-pronged. As fat is ingested, digested to FA and then incompletely absorbed by the upper intestine, they enter the ileum and enteroglucagon is released. Enteroglucagon then suppresses intestinal motility and helps maintain residual absorptive function in the short term by this slowing of transit time. Secondly, it may go on to stimulate residual bowel adaptation in the long term. Thus, it appears that fatty acids play a crucial role in inducing the adaptive process, probably through enteroglucagon, following intestinal resection. Further investigations into the direct effect of enteroglucagon on mucosal adaptation will help to confirm this theory.

To summarize, following massive resection, the bowel is able to compensate for the loss of absorptive surface area. This compensatory response is likely due to a combination of villous hypertrophy to some extent along with luminal dilatation. The degree to which the bowel can adapt, however, is multifactorial. Essentially, ileum has more capacity to adapt than jejunum and regulation of this adaptation is multifactorial including luminal nutrition, pancreatico-biliary secretions and hormonal influences.

Absorptive Function Following SBT

Once the function of greater than 75% of the small bowel is lost, usually due to surgical resection, the residual bowel is overwhelmed in its effort to absorb sufficient nutrition. Adaptation does occur in the remaining bowel as discussed above and reaches its maximum at one to two years.[39,200] This adaptation is usually inadequate to return bowel function to normal, simply because there is not sufficient length of bowel to re-establish sufficient surface area despite compensatory villous hypertrophy and luminal dilatation, hence the term "short bowel syndrome". While parenteral nutrition has reduced the mortality associated with this condition[181], it has inherent drawbacks including cost, psycho-social effects and long-term venous access with its own complications.

Thus, the alternative of small bowel transplantation was developed in order to re-establish a functioning nutritional absorptive organ in these patients. However, in SBT, there are multiple factors which can affect its absorptive function. Among these are graft-host interactions, immunosuppressive agents, and the effect of the surgery itself, that is, the denervation and lymphatic disruption of the graft.

Much of the literature shows that many of the absorptive functions of the bowel at the cellular level are altered by SBT. However, it would appear that the graft is able to compensate for these defects on a more global level since most literature demonstrates that, overall, absorption of dietary nutrients is maintained to a sufficient degree that normal growth can still occur.

Functional characteristics have been studied in terms of bowel permeability[73,156,163] and mucosal chloride secretion. These studies have been carried out in isogeneic and allogeneic transplant groups. As well, the effects of cyclosporin (CsA), the primary immunosuppressive drug in SBT, have also been investigated in non-transplant groups.[161] While Holmes et al. were not able to demonstrate any increased glucose permeability in the absence of rejection[73], Sigalet showed increased mannitol permeability in isogeneic groups of animals.[163] Since mannitol permeates via trans-membrane pores, alteration of these pores appears to be due to the transplantation process itself.[161] d-Xylose and glucose absorption were not significantly altered in non-rejecting allografts.[73,98,141,145,202] However, these groups demonstrate an increased permeability to lactulose and ^{51}Cr-EDTA[161], which cross the mucosa via tight-junctions. CsA has also been demonstrated to increase the permeability via the tight junctions.[161] Thus, allografted small bowel appears to have defects in its tight junction function, but whether this is due to CsA, allotransplantation or the combination is not clear.

Denervation of the bowel, a necessary occurrence in SBT, may also affect graft function. It has been noted that secretion of chloride and water occurs in denervation models and this has also been noted in allografted bowel.[202] Due to loss of sympathetic innervation, junctional permeability may also be altered by denervation.[163]

In spite of these alterations in absorption at the cellular level, weight gain following transplantation appears to be maintained in the long-term.[136,163,177] Furthermore, studies have demonstrated that transplanted bowel eventually reaches normal absorptive function.[86,139,177] Short-term studies were not able to demonstrate any

morphological changes[98] to explain the graft's ability to overcome the alterations described previously. However, long-term studies showed that at 150 days post-SBT, villus height and crypt depth, along with lumen diameter were significantly increased when compared to controls.[86] As well, these adaptations occurred in denervated animals versus innervated controls. Thus, the graft appears to adapt to its new environment in a similar fashion to residual bowel following massive resection. Thus, although the grafted bowel has sufficient length, it would appear that, at least initially, the functioning surface area is reduced. This is likely due to the fact that relatively under-utilized ileum (in the normal animal) comprises the majority of the graft. However, it has been demonstrated by Kimura et al. that some proximal jejunum must be transplanted, essentially ready to function, for recipient survival. If ileum alone is transplanted, survival is poor.[83] In spite of this, it appears that adaptive increases in surface area are required before normal absorptive function can be achieved. In addition, re-nervation has been demonstrated at one year[175], which may improve the permeability defects noted previously if they are, indeed, due to denervation.

More specific to our studies, fat absorption appears to be maintained following SBT. Studies of absorption of fat-soluble vitamin A [59] show normal digestion and absorption even with CsA therapy after 20 days post-transplant. Other authors have demonstrated normal oleic acid absorption at 2-4 weeks.[156,163,170,177] Sarr et al.[145] demonstrated normal fatty acid absorption at one week using a high fat diet in a model of canine autotransplantation with interuption of lymphatics from the duodenal-jejunal junction ot the ileo-cecal valve. The delay in observing normalization of fat absorptive

function is thought to be due to the time required for lymphatic regeneration, although Sarr's results suggest a possible role of lympho-venous connections in maintaining fat absorption early post-SBT. Chylomicrons demonstrated in the mesentery of recipients at 14 days[152,153], along with lymphangiographic studies to be discussed later, have provided authors with morphologic evidence of a delay in lymphatic regeneration. However, the assumption has been made in the literature that once the lymphatics re-form, they return to normal function in the fat absorptive function of the graft. This conclusion has yet not been confirmed experimentally. One of the aims of our study was to examine this issue directly.

Fat absorptive function following SBT is also important with regard to the use of immunosuppressives. CsA, the current primary immunosuppressive drug, is administered orally as a lipid emulsion.[59,139] Thus, if fat absorptive function is impaired, presumably CsA absorption will also be reduced. deBruin et al.[27] along with Kirsch et al.[86] independently demonstrated normal CsA absorption following SBT when compared to controls. This would indicate that, along with normal fat absorptive function, CsA is also absorbed normally. However, they also showed that graft survival was better using an intra-muscular administration technique versus an oral regime. The reasons for these seemingly dichotomous results are not evident. Schindler et al.[150] have demonstrated the development of a fat malabsorptive state with the onset of rejection. They point to lower absorption of vitamin A esters as evidence for this reduced function. As a result, they raise the concern that once rejection has begun. orally administered cyclosporin will not be absorbed in sufficient quantities to combat the rejection. They also suggest that

using a marker of the fat malabsorptive state, such as vitamin A, may help to identify rejection in a non-invasive fashion.

In summary then, nutrient absorption appears to be maintained following SBT provided that rejection is minimized. The use of immunosuppressives such as cyclosporin, although causing absorptive alterations at the cellular level, do not appear to affect the overall picture of nutrient absorption with regard to weight gain and growth.

Lymphatic Regeneration Following SBT

The ultimate goal of small bowel transplantation is to allow the graft recipient to receive all their nutritional requirements, whether it be carbohydrate, protein, fat or vitamins, from enteral sources alone.

As discussed previously, traditional teaching has suggested that the lymphatics are required mostly for long-chain fatty acid absorption. Among these long-chain fatty acids are the essential fatty acids, linoleic and linolenic acids. Thus, good lymphatic function is central to the fulfilment of the SBT goal.

SBT, by its very nature, necessarily disrupts the lymphatics draining the bowel. Once the graft is placed, full bowel absorptive function cannot be restored, and therefore complete enteric nutrition is not possible, unless functional lymphatics re-form.

Studies in canine models of SBT [55,87] have demonstrated lymphatic regeneration through the use of radio-opaque or mesenteric injection of coloured dyes as early as 2 weeks and uniformly by four weeks. At four weeks, balance studies demonstrated normal dietary fat absorption.

Rat studies using isogeneic orthotopic models have been performed as well with no attempt at lymphatic re-anastomosis.[88,148,152,153,192] Coloured dyes as well as lymphangiographic studies were again used. Early regeneration was noted by eight days. Furthermore, Schmid[152,153] microscopically demonstrated chylomicrons in the donor mesenteric lymphatics at 10 days and their subsequent appearance in the recipient lymphatics by 14 days. He also showed that CsA inhibited this regeneration, thus suggesting that in the clinical heterotopic model, the second stage should be delayed until 2 weeks, when lymphatics have regenerated to sufficient degree that graft fat absorption will be possible.

The dog studies as well as the rat studies, with the exception of Schmid's work, essentially demonstrate *patency* of lymphatics which regenerate across the line of resection in the mesentery. Patency does not necessarily equate with function. While Schmid's studies demonstrate the conveyance of fat-carrying packages by these lymphatics, it is not clear whether the degree of transport approaches normal or not. Thus, the assumption that these visually demonstrable regenerative lymphatics function normally has not been directly tested in the literature.

Small Bowel Transplantation Models

Although bowel transplantation had been done very early in the twentieth century, experimentation in the field enjoyed a resurgence of interest with the development of immunosuppressive agents.

It was in this era, during which SBT became clinically possible, that models of SBT were developed in order to test graft physiology. One of the first of these models was described by Lillehei and associates in 1959.[103] Their studies in dogs were plagued by high death rates. The high mortality was due to their animal model; mongrel dogs do not allow for good genetic control, thus immunologic control between donor and recipient is also difficult to control.

Monchik and Russell[113] in 1971 had somewhat better success in developing a rat model. Although the technical requirements of a microvascular anastomosis were greater than in the dog, their use of F_1 hybrid rats allowed them to perform small bowel transplants and evaluate rejection and graft-versus-host disease under genetic control.

The descriptions of Monchik and Russell [113] provided the basis for the development of the rat model for SBT. Using the F_1 hybrids, they first irradiated the donors for 24 hours prior to their use. Presumably, this was to reduce the function of the large numbers of immunologic cells which necessarily accompanied an enteric graft in the gut-associated lymphoid tissue (GALT). The superior mesenteric artery was anastomosed to the aorta and the portal vein was joined to the inferior vena cava at the level of the renal arteries. The graft itself was placed heterotopically; after revascularization, the graft ends were brought out as stomas with the native gut left

intact. Once the recipients had demonstrated prolonged survival, the native gut was resected and the graft was placed in the enteric stream. This two-stage model had a high mortality of 21 - 25%.

Believing that part of this mortality was attributable to two highly stressful operations, Kort[92] modified the rat model and performed a one-stage, orthotopic transplantation in which the host gut was removed and the donor gut immediately place in the enteric stream. Unfortunately, he was unable to reduce mortality in this model. Koltun[90] and others modified the heterotopic model by performing the gut resection and donor anastomosis at three weeks post-transplantation. Although the rats were again exposed to two surgical stresses, they were able to demonstrate reduced mortality.

As experience in the model has increased, technical modifications have been made which have improved mortality appreciably. It may be that mortality of early models was due in large part to the technical requirements of a microvascular anastomosis and as these techniques have been refined with time and experience, the survival has improved accordingly. This appears to be the theme of a paper published by Zhong and associates[211] describing improved survival in both orthotopic and heterotopic with careful microvascular technique combined with sufficient crystalloid for volume replacement. A 90% survival for heterotopic models demonstrated a remarkable improvement over previous models. Most significantly, however, was a long-term survival of 86% using their refined orthotopic technique. This demonstrated that a less stressful one-stage transplant was not only much more feasible than in the past, but also had a survival comparable to the heterotopic models. The orthotopic technique was

obviously more efficient in terms of time than the heterotopic models. Thus, although heterotopic transpants are still performed, especially in large animal models, orthotopic transplants have become the most widely-used rat model in recent years.

Due to decreased costs, good immunologic control and development of improved techniques, the rat now serves as the standard for initial investigations of SBT and related graft function and immuno-modulation.[162]

The Portal Drainage Controversy

More recently, controversy has arisen regarding the venous drainage of the graft. The portal-IVC anastomosis essentially creates a porto-systemic shunt and thus, concern regarding development of hepatic encephalopathy has been expressed. Altered levels of ammonia and amino acids similar to those found in hepatic encephalopathy models[89,90] have been demonstrated. The portal-IVC anastomosis has also been shown to alter vitamin A and zinc absorption[149]. With these early findings, many authors believed that a portal-portal anastomosis would be required in the clinical setting to avoid development of encephalopathy in SBT recipients. Furthermore, Schraut demonstrated an improved long-term survival using a portal-portal anastomosis. He concluded that this anastomosis must convey an immunologic advantage of some kind to the recipient.[155]

Since those early studies, however, numerous authors have been unable to show any difference in a wide variety of parameters between the two venous drainage routes, including weight gain, survival, and levels of amino acids and ammonia.[81,104,147,161] Specifically, Kaneko[81] was unable to demonstrate any evidence of deleterious metabolic

effects of a portal-IVC anastomosis in pigs. The rationale for performing these studies was possible differences between rats and humans in handling systemic drainage of the portal system, thus necessitating evaluation in a large animal model. Kaneko's results may indicate that human subjects will also be able to compensate for a porto-systemic shunt; this is probably because the liver is healthy in these subjects and is able to maintain normal ammonia and amino acid levels through more extensive extraction of these substances from the systemic circulation. However, liver disease following long-term TPN is well documented. Patients in this situation may be at greater risk for developing hepatic encephalopathy following systemic drainage of their small bowel graft. More study is required to evaluate this possibility.

Due to its technical ease versus portal-portal anastomosis and multiple studies showing no metabolic alterations, porto-caval drainage has been validated as an investigative model in SBT.[81,104,147] This method also has more clinical relevance as well: most patients requiring SBT will have undergone multiple abdominal operations with a high likelyhood of developing extensive adhesions. In this setting, a porto-portal anastomosis may be exceptionally difficult. Thus, a porto-caval anastomosis may be required and may, in fact, become the standard route of drainage as the techniques of clinical SBT evolve.

Fat Absorption Investigative Techniques

In order to assess the role of the enteric lymphatic drainage in fat absorption, it is necessary to gain access to the lymph itself and then measure the fat components of that

lymph. The first description of lymphatic cannulation techniques was published by Bollman and associates in 1948[14]. In this technique, the mesenteric duct is identified as it runs along the superior border of the superior mesenteric artery. The duct is incised transversely and a small silastic catheter is placed in the lumen. A silk suture then secures the cannula in place.

This technique has been modified somewhat by Tso [189-191] in that the difficult process of securing the cannula with silk suture has been replaced by the use of histo-acryl glue. This gives good fixation of the cannula in the lumen but has two further advantages: avoidance of excessive mobilization of the mesenteric duct and thus potential damage to the duct or surrounding structures, and the ability to re-position the cannula intra-operatively if obstruction occurs by removing the hardened glue and re-applying a second droplet for fixation after re-positioning the cannula.

During experiments on bowel lymphatics, this cannula is allowed to drain spontaneously[8,13,167,189-191] otherwise clotting occurs. Thus, many studies have been done on the rate of flow of lymph and factors affecting flow. Initial studies in 1953 by Simmonds showed that constant high-volume intra-duodenal infusions of water alone produced a transient increase in protein-poor lymph flow.[165] Normal saline seemed to induce a more prolonged rise in lymph flow with a better protein content. Of note for our study was his description of the higher, prolonged output of lymph in response to high dose triglyceride or other fatty compunds. Mansbach and Arnold also demonstrated increased TG output in the lymph with chronic feeding of a 20% fat diet.[106]

In 1966, Barrowman and associates[6] confirmed the findings of Simmonds by demonstrating a rapid response to water administration which is short-lived and a later response to saline with more prolonged increased flow. Their investigations led them to conclude that the lymphatics act as an early overflow route during intestinal absorption of hypotonic solutions. No investigations into the effects of fat administration were performed.

Tso et al.[189] then looked at how lymph flow affects chylomicron transportation and therefore fat absorption. They found that well-hydrated rats had much better lymph flow than dehydrated rats when a constant fat emulsion was administered intra-duodenally. They further demonstrated a longer appearance time of chylomicrons containing radio-labelled fat with lower lymph flow rates. They therefore concluded that a steady-state lymph flow must be achieved in order to study the absorption of fat via chylomicron formation. A study published later by Tso's group[191] demonstrated a progressive increase in lymph flow with a constant infusion of fat following saline infusion. By eight hours, a steady state of lymph flow was reached. Although the rate of flow did not differ appreciably between groups getting different doses of fat, the actual percent recovery in the lymph of continuously administered radio-labelled fat did increase with higher doses of fat. These findings confirmed the earlier results of Mansbach and Arnold.

These studies show that achieving a steady state lymphatic flow is mandatory in studying fat absorption in the conscious rat. Furthermore, the infusate components are

equally important in establishing a physiologic model of fat absorption. The work of the above investigators provided a methodologic basis for our own study.

In summary, the process of fat absorption has still not been completely elucidated under normal physiologic circumstances. It is reasonable to expect that by its very nature, small bowel transplantation will have some effect upon the pathways of fat absorption; if not at the cellular level, then at the macroscopic level. Early studies have suggested that fat absorption is maintained in the long term following SBT, but objective evidence for the mechanisms by which this occurs is lacking. One of the objectives of our study was to directly assess the effect of SBT on fat absorption by evaluating the function of the routes available for fat absorption, and thus provide insight into how the maintenance of fat absorption is achieved.

Chapter III - Experimental Materials and Methods

These studies were performed using the male Lewis rat. As mentioned previously, the rat model of SBT has become the standard for initial investigation of graft physiology. Lewis rats, being an inbred strain, allow for good genetic control. Thus, we could perform isogeneic SBT with confidence that rejection would not be a confounding factor in studying the physical effects of SBT on graft physiology.

All animals were purchased from Charles River, St. Constant, PQ and housed in individual Plexiglas cages in the vivarium of the Surgical-Medical Research Institute, University of Alberta. Three groups of animals were studied: controls, which underwent sham laparotomy, transplants, which underwent one-stage orthotopic SBT, and a group of un-operated rats which underwent cannulation only. This un-operated group which we refer to as "normals" was assessed in order to validate our testing methods for the other two groups.

Throughout these studies, the guidelines of the Canadian Council of Animal Welfare were followed in caring for the animals. Furthermore, due to the fact that our studies necessitated the use of radio-pharmaceuticals, the Radiation Safety Protocols of the University of Alberta were also followed. The protocol for these studies was approved for implementation by the Animal Welfare Committee of the University of Alberta.

Control Model

This group consisted of nineteen animals. Each was approximately 300 g prior to operation. The sham laparotomy was performed as follows.

Rats were fasted for 12 hours prior to the operation. Anesthesia was induced using 4% Halothane in an induction chamber. Animals were then transferred to a warmed stainless steel operating platform and maintenance anesthesia of 1% Halothane with 3 lpm $O_2$was administered by mask. The abdomen was shaved and prepared with a dilute Betadine solution. Cefazolin 25 mg/kg was administered subcutaneously prior to incision.

Under the operating microscope, the abdomen was opened through a long mid-line incision. The intra peritoneal small intestine and colon were then drawn out of the abdominal cavity and wrapped in warm saline-soaked gauze.

The retroperitoneal distal duodenal attachments were then incised to free up the duodenum. Approximately one centimetre distal to the duodenal-jejunal junction, the mesentery immediately adjacent to the bowel wall was perforated using a number 7 Duhamel forceps. The small mesenteric vessels on either side were ligated with 6-0 silk. The bowel was divided. Excessive bleeding was not encountered in any of the animals. The bowel was then immediately re-joined using 6-0 silk in an interrupted fashion. A macaroni noodle was used as a stent for the bowel anastomosis as described by Zhong[175].

The ileo-cecal junction was then defined. Approximately one centimetre proximal to this point, the mesentery was again perforated immediately adjacent to the

ileal wall. After ligating the mesenteric vessels, the bowel was again divided and re-anastomosed, again using a macaroni stent and 6-0 silk interrupted sutures. The bowel was then replaced in its normal position in the abdominal cavity. When possible, omentum was loosely wrapped around the anastomotic sites to buttress them against any leakage.

The abdominal wall was then closed in a single layer using a continuous 4-0 polyglycolic acid suture. Just prior to completing the abdominal closure, 4 cc of sterile normal saline were instilled into the cavity. Three mL of normal saline were placed subcutaneously and 2 ml were infused intravenously via the penile vein. Buprinorphine was also administered subcutaneously: 0.1 mg/kg.

The Halothane was discontinued and oxygen administered by mask at 5 lpm until the rat became wakeful. The animal was then placed in a cage under warming lights for twenty-four hours post-operatively.

Water was given *ad lib* once the animal had become active. Cefazolin 25 mg/kg was administered subcutaneously every 8 hours for three doses and buprinorphine 0.1 mg/kg was administered s.c. every 12 hours for 2 doses. At 24 hours, the animals were re-assessed, and if they were well-recovered, standard rat chow (Tekland Premium Lab Diet, Textron, Corp., Madison, WI) was given *ad lib*.

Out of nineteen rats undergoing this operation, we had only one early post-operative death. This occurred with our tenth control rat. This particular animal had been extremely agitated in our vivarium during the week prior to its operation. The operation went well with no blood loss. Within 24 hours, the animals ears were

extremely pale. Suspecting a gastrointestinal hemorrhage, subcutaneous fluids and ranitidine 0.4 mg were aggressively started. However, within 72 hours, the rat was found dead in its cage. At necropsy, massive GI hemorrhage was confirmed as the cause of death.

The remaining 18 rats demonstrated no ill effects from this operation. Control survival for sham laparotomy was therefore 95%.

Small Bowel Transplantation Model

Sixteen male Lewis rats weighing approximately 200-250 g were used for both donors and recipients. Following overnight fasting, animals were induced and maintained with Haothane and O_2 by mask, the rats were placed supine under the operating microscope.

A one-stage orthotopic translant was performed using the methods described by Zhong, et al.[211] The donor abdomen was opened and the entire small intestine was isolated on a pedicle containing the superior mesenteric artery and the portal vein. The bowel was divided 1 cm below the duodenal-jejunal ligament and 1 cm above the ileo-cecal valve. Heparin 150 units was then administered intravenously to provide systemic anticoagulation.

The aorta was then ligated above and below the origin of the superior mesenteric artery and the artery was then flushed with 2 - 3 mL of iced Ringer's lactate until the bowel blanched. The portal vein was ligated close to its bifurcation and allowed to drain

the blood forced out by the arterial infusion. The bowel was then quickly removed by cutting the mesentery along its root. The graft was then stored in iced Ringer's lactate.

The recipient rat was then opened through a mid-line incision. Again, the infra-renal aorta was isolated, as was the infra-renal inferior vena cava. The superior mesenteic artery and portal vein were also isolated, ligated and divided. The native small bowel from the duodenal-jejunal ligament to the ileo-cecal valve was then removed from the abdominal cavity.

The graft was then placed in the recipient's abdomen and revascularized through end to side anastomoses of the graft portal vein to the isolated infra-renal IVC and the superior mesenteric artery to the isolated infra-renal aorta. Monofilament 10-0 sutures were used for the vascular anasotmoses. Primary anastomoses of the two graft ends were then performed using 6-0 silk interrupted sutures and a macaroni noodle as a lumenal stent.

The abdomen was then closed using 4-0 polyglycolic acid sutures. The animal was recovered under warming lamps and once active, they were allowed free access to water. They were carefully followed for 48 hours post-operatively, and if doing well, were then given rat chow *ad lib*.

A single post-operative death occurred within 24 hours secondary to vascular complications. The operative survival in this group was 94%. As such, 15 rats consituted this experimental group.

Post - operative Follow-up

Both groups of rats were followed for six weeks following their initial operation. During this time, they were weighed weekly. Their food intake was also measured each week. In this way, we were able to have some assessment of growth to compare between groups. During the fifth post-operative week, the rats underwent metabolic studies in standard rat metabolic cages. Standard rat chow was replaced with a powdered food which had a fat content of 9.28% and consisted primarily of long-chain fatty acids.

The rats were placed in the metabolic cages for three days in order to acclimate them. Following this, their food intake and stool output were measured daily for three further days. The rats were then removed from the metabolic cages and replaced in their standard cages. The fat excreted in the stool was analyzed using the method of Fulch.[46] Dietary fat absorption was then calculated directly.

Cannulation Procedure

As discussed previously, many studies have shown that physical regeneration of lymphatics across the line of resection is optimal at four weeks.[55,87,152,153] Thus, we decided that a period of six weeks post-operatively would ensure that good lymphatic regeneration had occurred. Furthermore, the attributing of normalization of fat absorptive function on these regenerative lymphatics at four weeks in the literature, we felt, could be adequately tested at six weeks and would allow for slower regenerative processes in some animals.

Thus it was that we brought all the animals in both groups back for cannulation six weeks following their original operation. Animals were not fasted prior to this operation in order to ensure that the mesenteric duct would be distended and therefore, easier to cannulate. Corning© silastic catheters of various sizes were utilized .

Cannulation of Normal and Control Groups (Figure III-1)

Animals were again induced with Halothane in an induction chamber. They were then maintained using Halothane via mask at 1% and O_2 at 3 lpm. The neck and abdomen were shaved and prepared with Betadine solution. Cefazolin 25 mg/kg was administered subcutaneously.

The animals were immobilized supine on a warmed operating platform under the operating microscope. A transverse incision was made in the right neck centered on the head of the clavicle. The incision was carried down through the superficial muscular layers until the lower portion of the carotid triangle was entered. Blunt dissection was used to isolate the internal jugular vein from its surrounding structures. 6-0 silk ligatures were placed above and below the cannulation site and the upper jugular vein was tied off. A transverse phlebotomy was made using fine iris scissors. A catheter measuring 0.062" inside diameter (I.D.) was then inserted into the lumen of the vein. The catheter was advanced approximately 5 cm into the proximal vein. The lower ligature was then tied around the vein and the catheter within in order to secure its position. Flow was assessed using rapid aspiration with a 5 cc syringe containing heparinized saline (1 i.u. per ml).

Following insertion of the jugular vein catheter, the abdomen was moved into the operative field. The abdomen was opened via a mid-line incision, utilizing the old incision in control animals. Retractors were placed to hold back the abdominal wall. The bowel was wrapped in warm saline-soaked gauze following its rotation out of the abdominal cavity. The proximal mesenteric duct was isolated in the retroperitoneum just above the superior mesenteric artery, medial to the inferior vena cava. A transverse cut was made in the duct and white lymph identified flowing from the incision. A catheter measuring 0.020" I.D. with a bevelled end was inserted into the duct and, once positioned, was held in place using a drop of Histo-acryl© glue. A small amount of heparinized saline was injected via the catheter into the duct to prevent early clotting of the lymph. The syringe was then removed from the end of the catheter and lymph was allowed to drain freely.

Attention was then turned to the portal vein. A constant vessel was noted emerging from the adjacent pancreatic tissue in all rats approximately 5 mm below the bifurcation of the portal vein. This was vessel was identified as the coronary vein, and it was used as our route of portal vein cannulation. Again the coronary vein was dissected free of its surrounding tissue. Unfortunately, it was difficult to free more than three to four millimeters of its length from its junction with the portal vein due to fine vessels in the pancreatic tissue which bled vigorously if disturbed. A 6-0 silk ligature was placed around the vein at the furthest point of isolation away from the portal vein and tied. Another 6-0 silk suture was placed around the vein almost at its confluence with the portal vein but was not tied. Tension was placed on both of these ligatures and a

transverse phlebotomy was made. A 0.020" I.D. catheter with bevelled tip and distal side hole was then inserted into the vein and passed into the upper portal vein itself. The catheter was positioned with its tip at the bifurcation and placement was confirmed using small injections of heparinized saline and observing the site of turbulent flow created at the catheter's tip. The catheter was then secured using the untied ligature. To ensure fixation, a drop of histo-acryl glue was placed on the catheter entrance site.

A purse-string suture was then placed in the anterior wall of the stomach. A small hole was made in the middle of this purse-string with cautery. A 0.062" I.D. catheter was then placed through the opening and advanced 2 cm into the duodenum. The purse string was tightened and tied. The bowel was then returned to the abdominal cavity.

A small incision was then made in the skin over the right flank. The jugular line was brought from the neck incision through a subcutaneous tunnel and out of the skin incision. The three intra-abdominal catheters were brought out through stab wounds in the right flank musculature and through the skin incision.

The abdominal incision was then closed using 4-0 polyglycolic acid after infusing 4 cc of normal saline into the peritoneal cavity. The neck incision was similarly closed. The blood lines were heparin-locked for transfer of the animal into the immobilization cages.

Transplant Cannulation Procedure (Figure III-2)

The transplanted rats had jugular lines inserted exactly as above. Furthermore, the mesenteric duct was cannulated and the duodenal tube were also inserted in a similar

fashion. Due to the portal-infra-renal-IVC anastomosis, the portal vein required a retrograde cannulation. To do this, the right lumbar vein was isolated in the retroperitoneum and tied off distally. A transverse phlebotomy was again created. The curvaceous nature of the route, coupled with the need to accurately control the rotation of the catheter into position, required creation of a stiff, curved catheter. For this, we used 0.020" I.D. polyethylene tubing. The tubing was heated approximately 5 mm from its end and slowly bent to create a sixty-degree curve while not compromising the lumen of the cannula. The tip was then bevelled and a small side hole was created near the end as well. The catheter was then inserted into the lumbar vein and passed into the lower IVC. The tip was then manipulated using external pressure and catheter rotation until it passed the portal-IVC anastomosis. The catheter was then advanced until the tip lay 6 mm above the anastomosis in the portal vein. Position was again confirmed using heparinized saline flushes and identifying the site of turbulent flow at the tip.

All lines were brought out through a right flank incision as in the control animals. After replacing the bowel and instilling 5 cc normal saline into the abdominal cavity, the abdominal and neck incisions were closed. The lines were again heparin locked for transfer.

Figure III-1
Fat Absorption Following SBT:
CONTROL MODEL

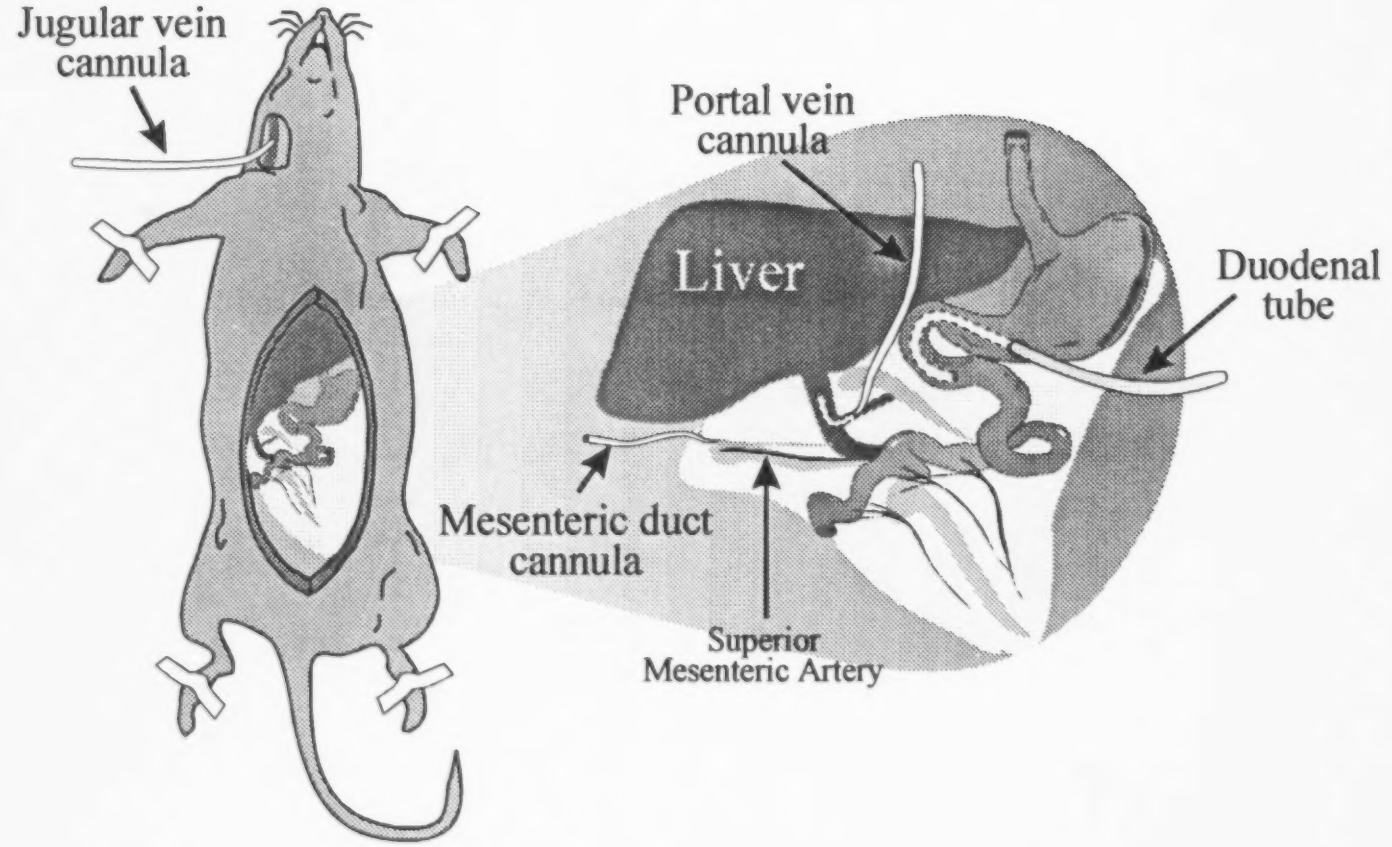
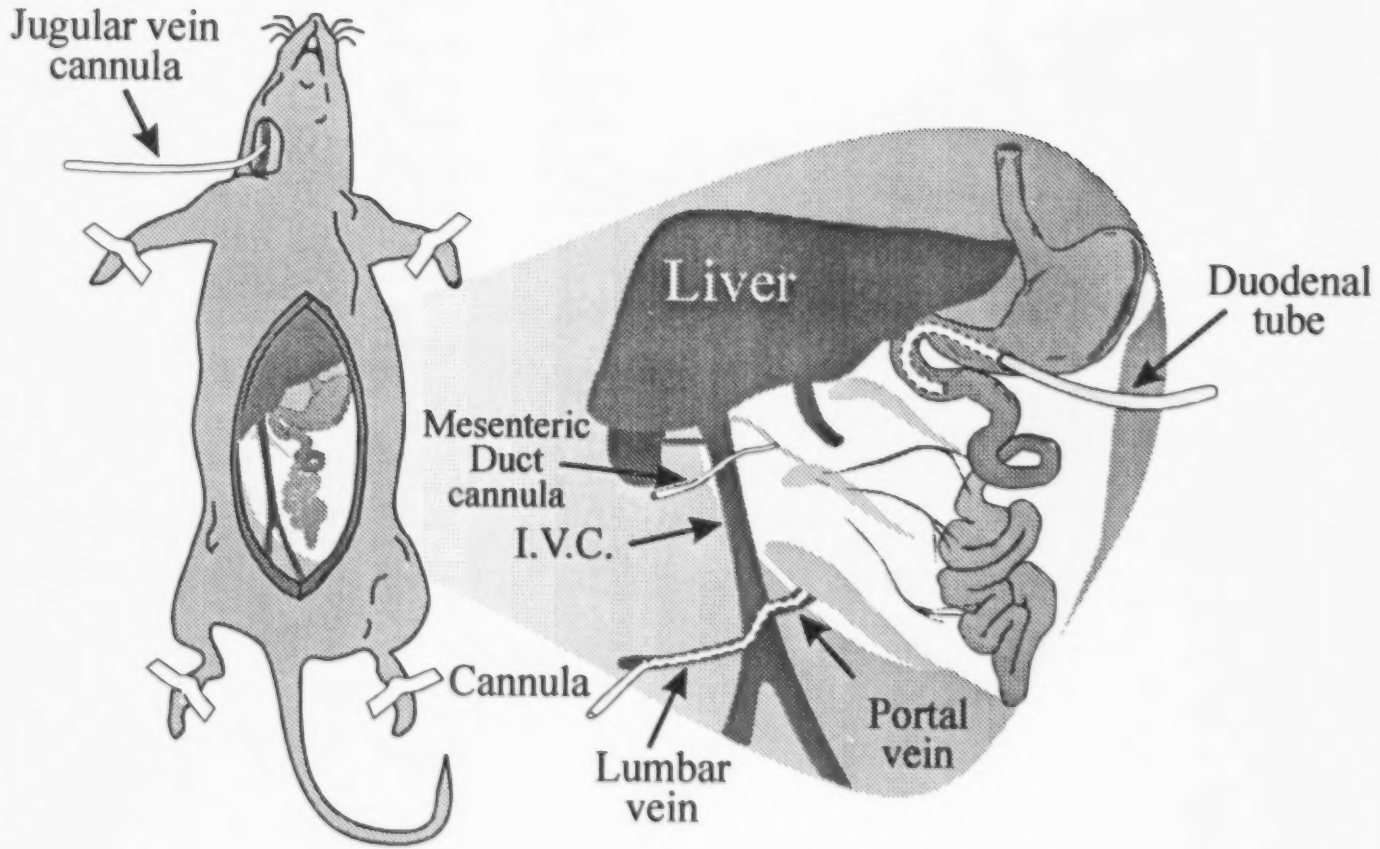


Figure III-2
Fat Absorption Following SBT:
SBT MODEL



Post-cannulation care - all groups

The animals were brought out from anesthesia using oxygen by mask at 5 lpm. In order to prevent dislodgment of the catheters, the animals required immobilization in Bollman cages. Although conscious, sedation consisting of buprinorphine 0.15 mg/kg and diazepam 0.5 mg/kg subcutaneously every 3 to 4 hours was administered starting in the immediate post-operative period. Ambient temperature was maintained at 26 - 30 degrees Celsius using an overheat incubator heat source. The blood lines were attached to syringes containing heparinized saline (2 i.u. per ml) which was infused at 2 ml per hour through both jugular and portal lines using a Harvard© syringe pump. Additional fluid was administered every 6 hours by subcutaneous injection 5 cc at a time.

Due to the stress involved in the immobilization, ranitidine was also administered in doses of 1 mg every 8 hours by slow intravenous push, in an attempt to reduce gastrointestinal stress hemorrhage which would also compromise fat absorption.

A fat emulsion was administered through the duodenal tube immediately following recovery of the animal. The components of the emulsion were based on the extensive fat absorption work published by Tso and associates[189-191]. The emulsion comprised two fatty acids: a medium chain FA, lauric acid (C12:0), and a long chain FA, palmitic acid (C16:0) (Sigma Chemical©). 40 μmol of each fatty acid were present per 3 ml of emulsion. Essentially, this resulted in a 0.3% solution of lauric acid and a 0.37% solution of palmitic acid administered intraduodenally. Conversion of this percent fatty acid solution figure to a dietary intake total is difficult but it probably represents a low to moderate fat load. Homogeneous dissolution of the fatty acids was brought about

by using the biological detergent egg lecithin (Sigma©) and the bile salt taurocholic acid (Aldrich Chemical©). The amounts of these components had to be altered from Tso's descriptions due to the difficulty in evenly distributing the radio-labelled fat throughout the sample to be administered for testing; it was therefore not possible to accurately measure the amount of radio-labelled fat the animal received. To improve this situation, we increased the amount of lecithin and taurocholic acid by 25%: 9.75 μmol lecithin and 71.25 μmol taurocholic acid per three ml of emulsion. Following this method change, retesting of samples demonstrated much more consistent counts between multiple aliquots of the same sample: less than 1% difference between three aliquots was measured on five individually prepared test emulsions (data not shown). The fat and detergents were mixed in the above amounts per three ml phosphate-buffered saline (PBS) (Na_2HPO_4 - 6.75 mmol, NaH_2PO_4 - 16.5 mmol, NaCl - 115 mmol, KCl - 5 mmol, in one litre double distilled water). The PBS has a pH of 6.4 which approximates the duodenal pH. The emulsion essentially formed a mixed micellar solution. Although not purely micellar, we were still able to simulate the post-digestion composition of duodenal chyme, thus presenting fat to the intestinal mucosa in a readily absorbable form. The fat emulsion was started immediately post-operatively and continued at a rate of three ml per hour via a separate syringe pump throughout the experiment.

Upon beginning the fat administration, the freely-flowing lymph from the mesenteric duct cannula was collected in tubes which were changed hourly in order to accurately measure lymph flow. As discussed previously, Tso[189] had shown that stable lymph flow was required in order to accurately assess fat absorption. Stabilization of

flow in all groups in our study occurred between 5 and 7 hours following the start if the fat infusion. This correlated well with Tso's work.

Once the lymph flow had stabilized, we administered a bolus of radio-labelled fat. Specifically, 20 μCi of ^{3}H-labelled lauric acid and 4 μCi of ^{14}C-labelled palmitic acid (Dupont©- New England Nuclear Products) were placed in a clean test tube. The ethanol solvent in which they were dissolved made homogeneous distribution in the sample difficult and also led to reduced absorption of the palmitic acid in pilot studies. Thus, the fatty acid aliquots were dried under nitrogen in a warm water bath. The nitrogen prevented volatilization of the lauric acid and oxidation of the dried forms of both fatty acids. Immediately, 1.2 ml of standard fat emulsion were added to the test tube and mixing was facilitated using sonication. Low intensity sonication applied in three twenty second bursts almost clarified the solution and gave the most homogeneous distribution of both fatty acid labels with multiple aliquots varying by less than 5% for both fatty acids.

The duodenal tube in each animal measured 40 cm in length, and having an internal diameter of 0.62", the lumenal volume was calculated to be 0.75 ml. A 0.5 ml aliquot of labelled fat solution was administered through the duodenal tube and flushed through with a further 0.5 ml of cold emulsion. Thus, we could be assured that all the label had been administered and we knew the precise moment at which the label came into contact with the absorptive surface of the bowel. At the same moment the labelled pulse was administered, the lymph line was changed over to a fresh tube to be collected.

A 100 µl aliquot of the labelled fat sample was also taken in order to exactly quantify the amount of radioactivity administered to the animal for each fatty acid.

Sampling Procedure

In order to precisely analyze the characteristics of fat absorption, we began experiments in normal male Lewis rats without previous operation. These animals numbered six. Keying on Simmonds' work[166] which suggested that fat absorption takes place primarily in the duodenum and upper jejunum, we chose to sample frequently in the first four hours following administration of the radio-labelled fat pulse and then hourly over a period of twenty hours as animal survival allowed.

Thus, we sampled in 100 µl aliquots from the blood lines every fifteen minutes over the first 2 hours and every 30 minutes for the second 2 hours. The lymph was collected in fresh tubes at the end of each fifteen minute interval for the first 2 hours and each 30 minute interval for the subsequent 2 hours and hourly thereafter. The quantity of each lymph sample was measured. A 100µl aliquot was taken from each lymph sample after mixing in a vortex for 10 seconds. All samples were placed in scintillation vials containing 3 ml of standard scintillation fluid. The samples were then counted for decays per minute (DPM) for both tritium and carbon-14 in a Beckman© scintillation counter.

With the results of the normal animals confirming that the majority of the label is absorbed in the first four hours, the control and transplant groups were subjected to a four hour sampling period in order to minimize the stress of immobilization. The four hour

sampling had the same frequency described for the initial four hours in the normal animals.

Following the sampling period, the animals underwent euthanasia with injection of one ml Euthanyl© via the internal jugular line. Following euthanasia, the positions of all catheters was checked by direct visual examination of the cannula insertion site.

Methylene Blue Studies

Prior to euthanasia, selected SBT rats were removed from their restraint cages and re-anesthetized. Following this, they were returned to the operative platform under the operating microscope and their abdomens were re-opened.

A lymph node immediately adjacent to the bowel in the mid-portion of the graft was identified and injected with 0.5 cc of methylene blue. The egress of the dye through the mesenteric lymphatics and into the systemic, recipient lymphatics was observed and the time for the dye to reach various landmark sites was carefully noted.

Carcass Studies

To further assess the absorption of fat and its distribution in the body, the normal animals, after their twenty-hour sampling, had their abdomens re-opened following euthanasia. The liver and bowel of each rat was removed, stored separately and frozen, as were the animal carcasses. The liver and bowel were then individually homogenized with an equal volume of 1 molar NaOH, and left at room temperature for 24 hours. The rationale for this procedure was to allow for cellular breakdown and release of fatty

acids. The tissues were then subjected to high frequency sonication and freeze dried. Fat extractions were undertaken on the tissues using the method of Fulch.[46] Aliquots of reconstituted samples were then analyzed for each radio-label in the scintillation counter. The carcasses of each animal were first autoclaved for three hours in order to soften the tissues and bones. They were then homogenized using a Warring blender and samples were taken from the homogenate. The samples were freeze-dried and again had Fulch fat extractions performed upon them. Reconstituted samples were again subjected to scintillation analysis.

Calculations, Comparisons and Statistical Methods

In assessing the fat absorption abilities of the bowel, we were interested in how the labelled fat was absorbed into the lymphatics and portal vein, and the subsequent appearance in the systemic circulation i.e. the jugular blood.

For the lymphatic absorption, we multiplied the number of DPM in each sample by the total volume of the sample from which it came. Thus, the total amount of absorbed labelled fat was calculated for that sample. We then added the totals for each sampling interval together and arrived at the total amount of labelled fat absorbed during the sampling period. This value was then expressed as a percentage of the total amount of labelled fat administered for each animal. Mean total absorption for the sampling period was calculated for each animal. The groups were then compared using a two-tailed Student's t-test with a level of significance of $p < 0.10$. For the normal group,

having fewer numbers, comparisons were made using the non-parametric Mann-Whitney U-test. A level of significance of $p < 0.05$ was used in this test.

The measured DPM for the jugular and portal blood for each sample interval were plotted on a curve using a pharmaco-kinetic computer model: PCNONLIN v. 4.2, SCI Software, 1992. This model assumes a constant volume of distribution for an absorbed substance and calculates the area under the absorption curve (AUC) which represents the total amount of the substance absorbed over the period of time studied. An additional feature of this program is its ability to calculate the blood absorption AUC of an orally administered substance thus making it ideal for our study. Pilot studies had shown that the portal and jugular curves converged at about 180 minutes following administration of the radioactive pulse. Thus, in order to minimize inclusion of re-circulated fat in our absorption calculation, we used jugular and portal samples taken for the first 3 hours only. The 3 hour absorption mean for each group was calculated for each fatty acid recovered from both portal and jugular lines. The means were then compared, again using a two-tailed Student's t-test with a level of significance of $p < 0.10$.

The mean fat extraction values for each labelled fatty acid in the liver, bowel and carcass of each animal in the "normal" group were also compared using a two-tailed Student's t-test, again with a level of significance of $p < 0.10$.

Chapter IV - Study Results

Growth and Metabolic Studies (Figure IV-1)

These studies were carried out on control and transplant animals only.

Control animals had a mean weight of 280g at the time of sham laparotomy. Over the subsequent six weeks, these animals gained a mean of 115 g ± 22g. They experienced a maximal weight gain of 29 ± 12 g during the second week following their operation. Growth then slowly plateaued toward the end of the observation period. Metabolic studies during the 5th week showed that these animals had an energy extraction of 81.8% ± 1.93. They also demonstrated a dietary fat absorption of 83.5% ± 1.55.

Transplant animals started at a mean weight of 261 g. Their post-operative growth pattern mirrored that of the controls; their peak growth period occurred in the second week with a weight gain of 21.7 ± 14 g and again slowly plateaued nearing the end of the monitoring period. However, these animals experienced weight loss to a greater degree than the controls during the metabolic cage phase of the study: overall weight gain during that week had a mean of 0.44g ± 23.9 g. Thus, the mean total weight gain for this group was 86.5 ± 32.6 g. The metabolic studies performed during the 5th week again demonstrated an energy extraction of 79.84% ± 2.04 and a total dietary fat absorption of 80.72 ± 1.94.

A Student's t-test was performed to compare the mean overall weight gain between groups. This demonstrated that the transplant group had a reduction in weight

gain at six weeks when compared to controls with a level of significance of $p < 0.01$. Again, this was due almost entirely to weight loss encountered by the transplanted group during the metabolic cage period of study. Student's t-tests were then performed comparing the mean energy extraction and dietary fat absorption between the two groups. No significant differences were demonstrated for these results.

Cannulation Studies

As stated previously, these studies tended to be quite stressful to the animals in spite of good sedation, pain control and a three day period of accustomization to the Bollman cage in the vivarium. Several animals in all groups experienced sudden death during the monitoring period. A cause of death could not be identified in many cases. Furthermore, catheter attrition was a major problem. Although all the animals in each group had the three sampling cannulas placed, the loss of any one or more of the lines due to clotting was not uncommon. Thus, the number of actual sampling lines is not the same as the total number of animals used.

Lymphatic Studies (Table IV-1)

The group of normals (n=6) served as the basis for defining the temporal relationships of fat absorption in our studies. As such, they underwent long-term monitoring following infusion of the radio-labeled fat, up to 20 hours. Four of the original six animals survived past the initial four hour observation period.

The mean hourly lymph flow for these animals was 4.5 ± 1.4 mL/hour over the full twenty hour period. In the first four hours, these animals absorbed 32.2 ± 10.1 percent of the administered lauric acid. They absorbed 35.6 ± 12.1 percent of the administered palmitic acid. By 20 hours, 51.0 ± 6.5 % lauric acid and 58.7 ± 6.4 % palmitic acid were absorbed by this group. Thus, 63% of the total lauric acid absorbed at twenty hours and 61% of the total palmitic acid absorbed at twenty hours was absorbed in the first four hours of the study period. (Figure IV-2)

The control group of nineteen animals yielded ten patent lymphatic lines for the full period of study. The mean hourly flow following stabilization was 4.8 ± 1.1 mL/hour. During the first four hours, these animals demonstrated that 33.3 ± 11.4 % of the administered lauric acid and 37.6 ± 13.8 % of the administered palmitic acid were absorbed. Four control animals were carried to twenty hours in order to demonstrate consistency of this group with the normal group. These four animals absorbed 43.5 ± 5.5 % of the lauric acid administered and 49.7 ± 8.6 % of the administered palmitic acid via the mesenteric duct by 20 hours. Thus, in the first four hours, the control group absorbed 77% of the total absorbed lauric acid and 76% of the total absorbed palmitic acid. (Figure IV-3)

When comparing the normal and control groups, we discovered that, although the normals did have a higher total absorption of both lauric and palmitic acid, this did not reach statistical significance by Student's t-test. Furthermore, both groups demonstrated no significant differences in the amount of medium versus long-chain fatty acid absorbed via the lymphatics either in the four or twenty hour sampling periods.

Out of fifteen transplant animals, seven maintained patent lymphatic lines for the full four hour period of sampling. Following lymph flow stabilization, this group had an mean lymphatic flow rate of 1.0 ± 0.29 mL per hour. Lauric acid absorption via this route was 7.4 ± 2.5 % of total administered. Palmitic acid absorption was 7.4 ± 2.6 % of the total administered. No transplants were carried past four hours.(Figure IV-4)

In comparing the fat absorption via the mesenteric duct in the SBT animals to the control group, the substantial reductions in lymph flow rate and absorption of both fatty acids were indeed significant. Student's t-test for mean hourly lymph flow versus controls yielded a value of $p < 0.0001$. Furthermore, lauric and palmitic acid absorption versus controls both gave a value of $p < 0.0001$. However, we were again unable to demonstrate any difference in the amounts of lauric and palmitic acid absorbed via this primary lymphatic route.(Figure IV-5)

Jugular Venous Studies (Table IV-2)

As discussed in the materials and methods chapter, three hour absorption curves were constructed for both the jugular and portal samples due to convergence of the curves at this point.

The area under the curve (AUC) was converted to a percentage of the total amount of radio-label administered.

Normal animals (patent cannulas = 6) demonstrated 0.58 ± 0.23 % lauric acid appearance and 0.11 ± 0.02 % palmitic acid appearance in the jugular blood. Control animals (patent cannulas = 12) had 0.75 ± 0.55 % lauric acid and 0.41 ± 0.53 % palmitic

acid appearing in the jugular blood. Transplants (patent cannulas = 12) showed an appearance of 2.01 ± 1.1 % lauric acid and 1.45 ± 0.74 % palmitic acid in the systemic circulation. When these normals and controls were compared, no significant difference for either fatty acid was demonstrable. On the other hand, the appearance of higher amounts of both fatty acids in the jugular blood of transplanted animals was statistically significant with a value of $p < 0.05$ versus controls.

Portal Venous Studies (Table IV-3)

Six patent portal vein catheters were mainatained in normal animals. The amount of lauric acid appearing in the portal blood was 0.87 ± 0.20 % of total administered. Palmitic acid appeared in an average quantity of 0.15 ± 0.05 % in the portal blood of normal animals. Control animals demonstrated an appearance of 1.00 ± 0.55 % lauric acid and 0.43 ± 0.58 % palmitic acid in the portal circulation. Recovery of lauric and palmitic acids from the portal vein of the transplant group averaged 1.91 ± 1.1 % and 1.38 ± 0.86 % respectively.

Again, on statistical analysis, there was no significant difference in the recovered quantities of either fatty acid when comparisons between normals and controls were carried out. However, the transplant group again displayed that the elevated levels of both fatty acids in the portal circulation was significant when compared to controls, yielding a value of $p < 0.10$ for lauric acid and $p < 0.05$ for palmitic acid. Furthermore, we again were unable to demonstrate any significant difference in the appearance of lauric acid or palmitic acid in the portal circulation of either the control or transplant

groups. However, in the normal group, the much lower quantity of palmitic acid appearing in the portal circulation compared with lauric acid did reach a level of significance of $p < 0.0001$.(Figure IV-6)

Methylene Blue Studies

The flow of dye through the graft and recipient lymphatics was easily identified by the naked eye.

Dense staining around the arterial anastomosis was noted approximately 15 minutes following injection of the mesenteric lymph node. Also at this time, a substantial lymphatic accompanying the left lumbar vein was also noted in the retroperitoneum. (Plate IV-1)

The dye from these areas subsequently entered a large para-caval lymphatic running between the IVC and the aorta. The diameter of this lymphatic approximated that of the normal mesenteric duct of the control animals, about 1 mm. Joining this para-caval lymphatic at about 30 minutes post-injection was dye entering via the most proximal mesenteric duct. (Plate IV-2)

Thus, it appears that a connection between the para-caval lymphatic and the mesenteric duct normally exists but only becomes apparent following SBT.

Once joined by the mesenteric duct, this ascending lymphatic is seen to be entering the cisterna chyli.

Also of note was that the period of time required for complete wash out of the dye in the perianastomotic and retroperitoneal areas was approximately 30 minutes.

Plate IV-1

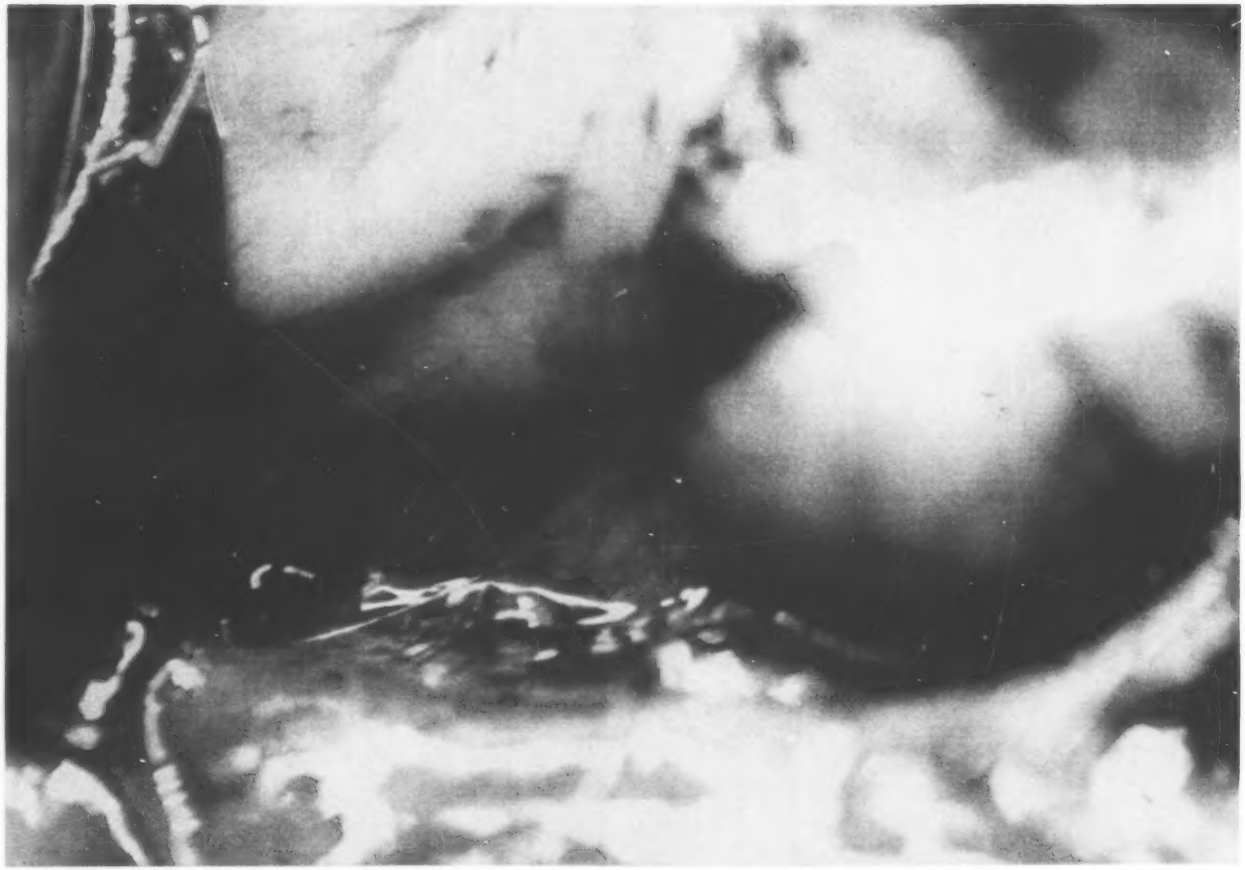
Regenerative Perianastomotic and Retroperitoneal Lymphatics

Methylene Blue Injection - 15 minutes

Plate IV-2

Proximal Mesenteric Duct and Its Junction with Paracaval Lymphatic

Methylene Blue Injection



Organ and Carcass Studies - Normal Group only

These studies were undertaken in the normal group only. They were done following the twenty hours of sampling following the administration of the radio-labelled fat in order to gain some understanding of the distribution of the absorbed fatty acids throughout the animal after a prolonged period of time.

The mean lauric acid recovery from the liver homogenate of these animals was $0.81 \pm 0.14\%$. Palmitic acid recovery from the liver had a mean of $0.95 \pm 0.15\%$.

The mean recovery of lauric and palmitic acids from the bowel homogenate averaged $9.92 \pm 1.23\%$ and $6.79 \pm 0.59\%$ respectively.

The carcass itself yielded a mean of 0.32 ± 0.10 % of the total lauric acid administered. Mean palmitic acid recovery from the carcass was $0.68 \pm 0.12\%$ of the total administered.

Figure IV-1
Mean 6 Week Weight Gain

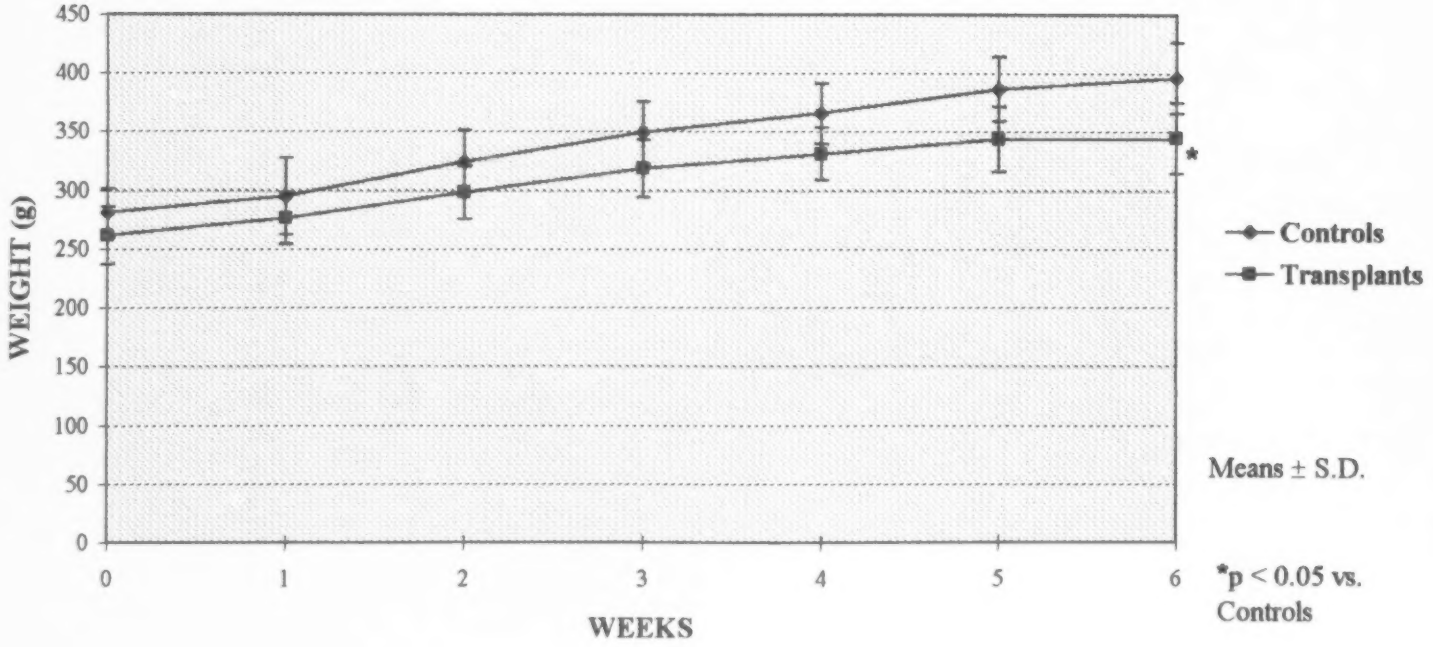


Table IV - 1

Mesenteric Duct Fatty Acid Absorption

			Percent Fat Recovered	
Group	***n***	**Flow (mL/hr)**	**Lauric Acid**	**Palmitic Acid**
Normals	6	4.5 ± 1.4	32.2 ± 10.1	35.6 ± 12.1
Controls	10	4.8 ± 1.1	33.3 ± 11.4	37.6 ± 13.8
Transplants	7	1.0 ± 0.29	7.5 ± 2.5*	7.4 ± 2.6*

Means ± S.D.

* $p < 0.001$ T-test vs. Controls

Figure IV-2
Mean Cumulative 20 Hour Lymphatic Absorption - Normals

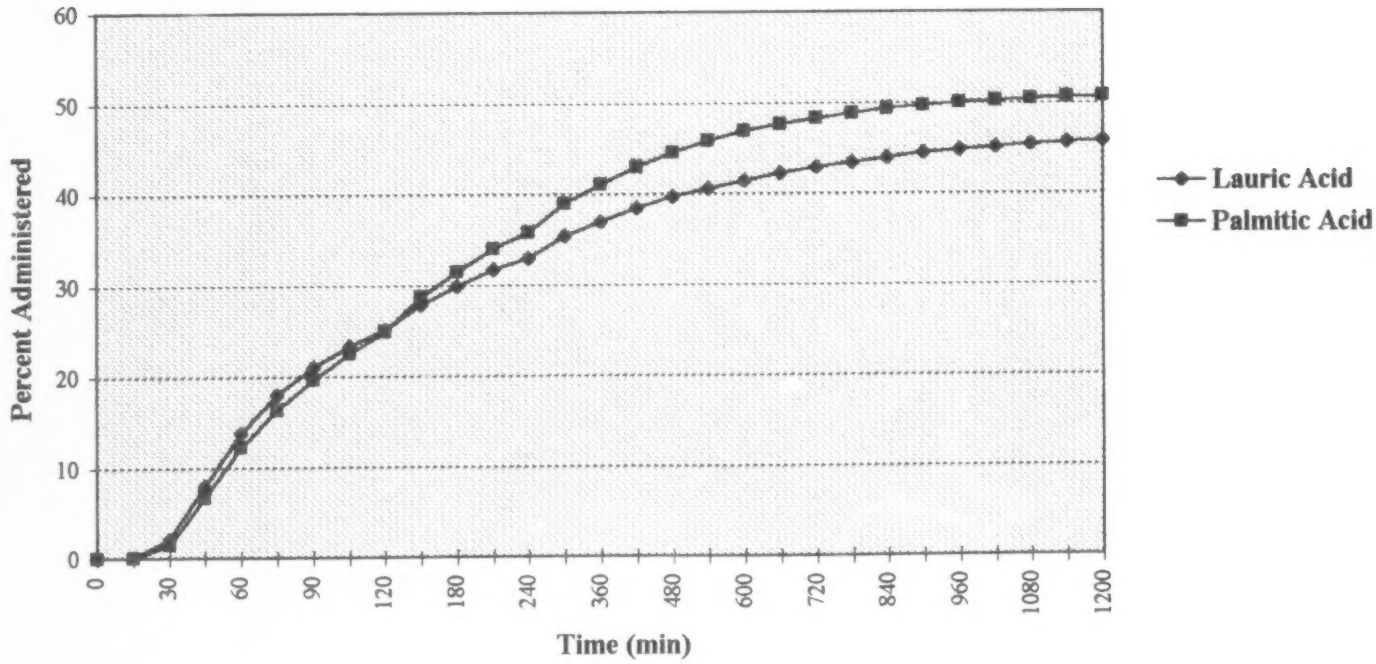


Figure IV-3
Mean Cumulative 20 Hour Lymphatic Absorption - Controls

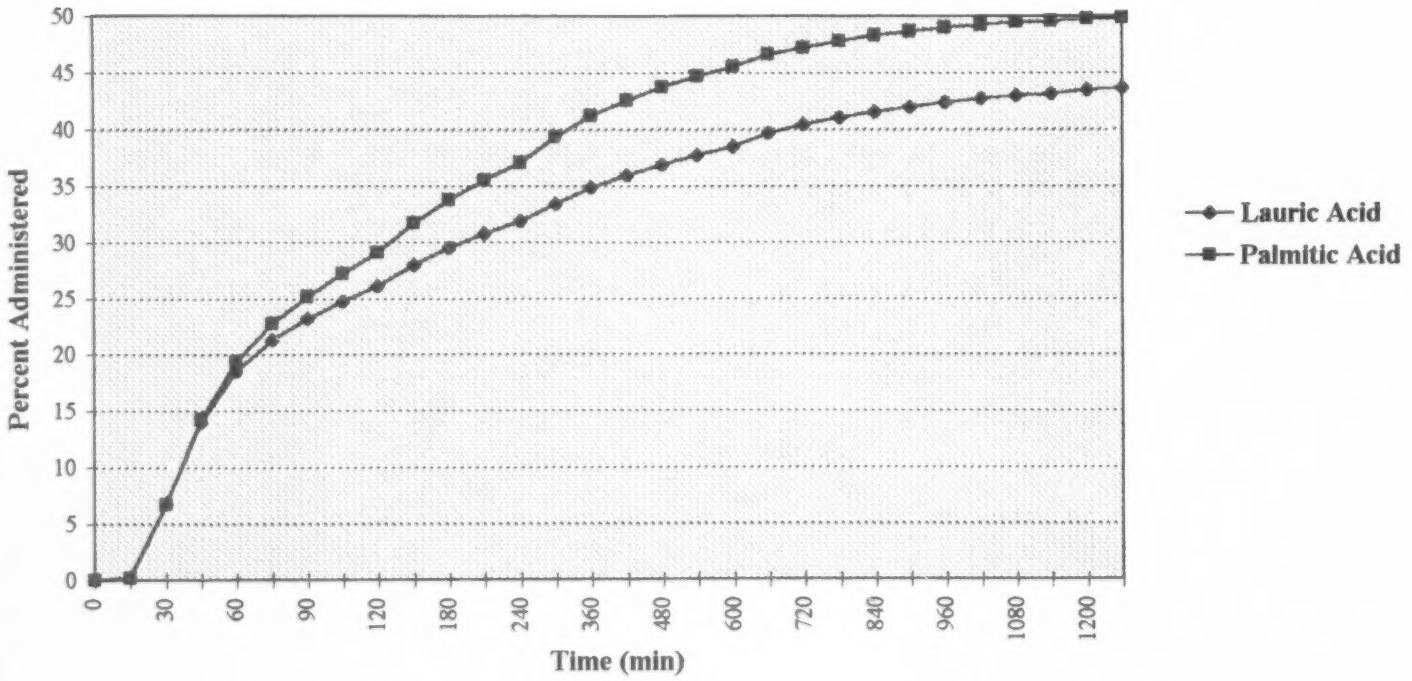


Figure IV-4
Mean Cumulative 4 Hour Lymphatic Absorption - Transplants

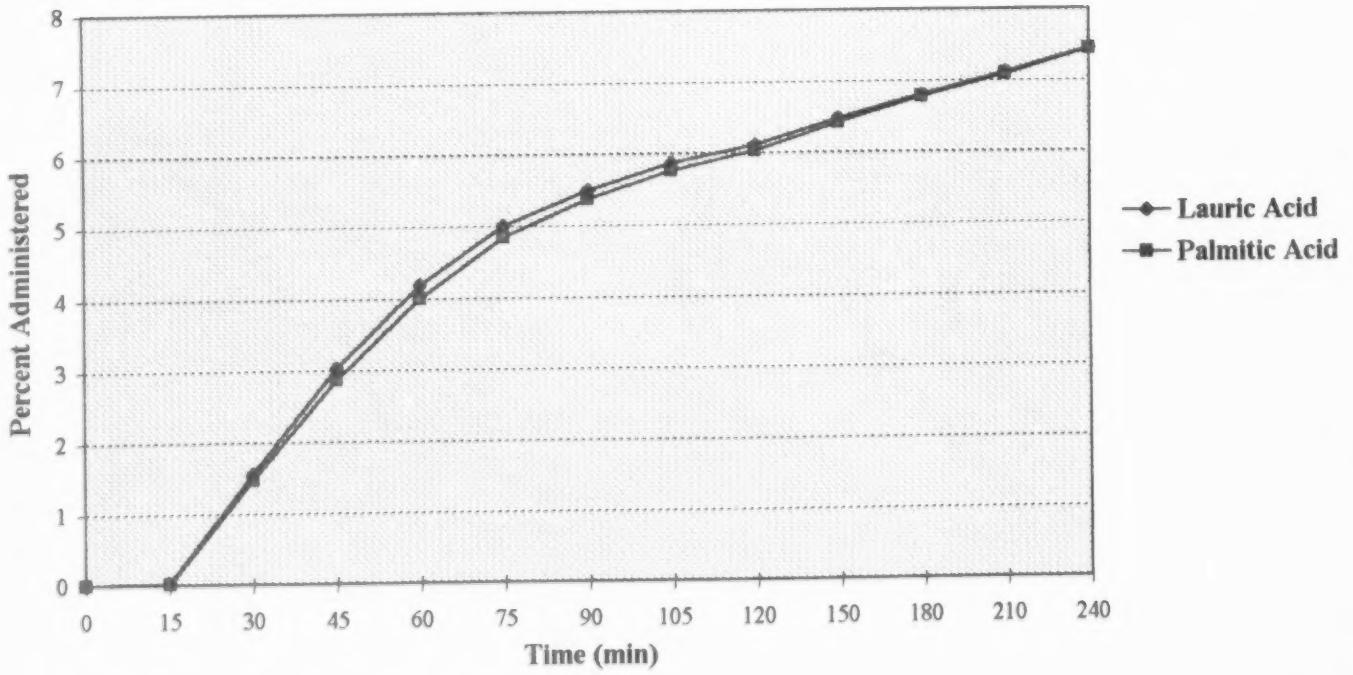


Figure IV-5
Mean 4 Hour Total Mesenteric Duct Fatty Acid Recovery

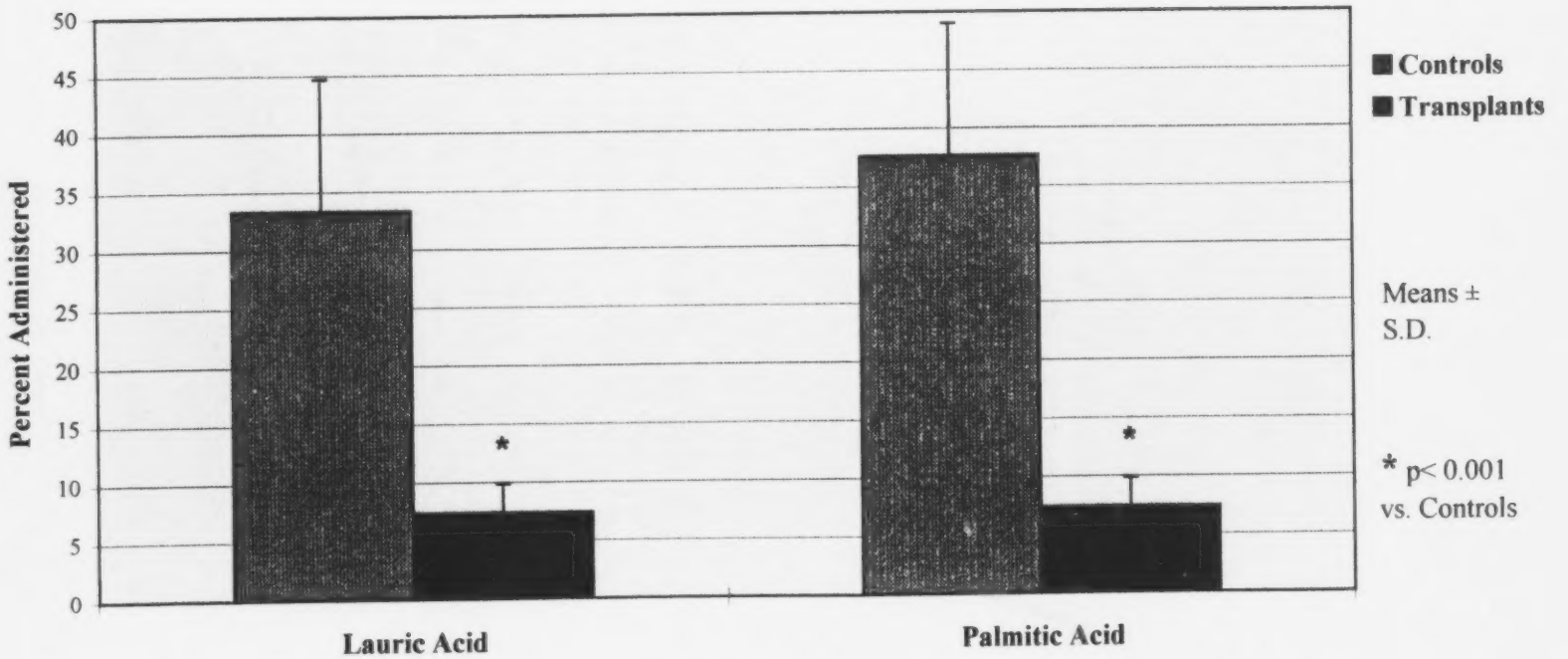


Table IV - 2

Jugular Blood Fatty Acid Recovery

		Percent Fat Recovered	
Group	***n***	**Lauric Acid**	**Palmitic Acid**
Normals	6	0.58 ± 0.23	0.11 ± 0.02*
Controls	12	0.75 ± 0.55	0.41 ± 0.53
Transplants	11	2.0 ± 1.1*	1.4 ± 0.74*

Means ± S.D.

* $p < 0.05$ *t*-test vs. Controls

Table IV - 3

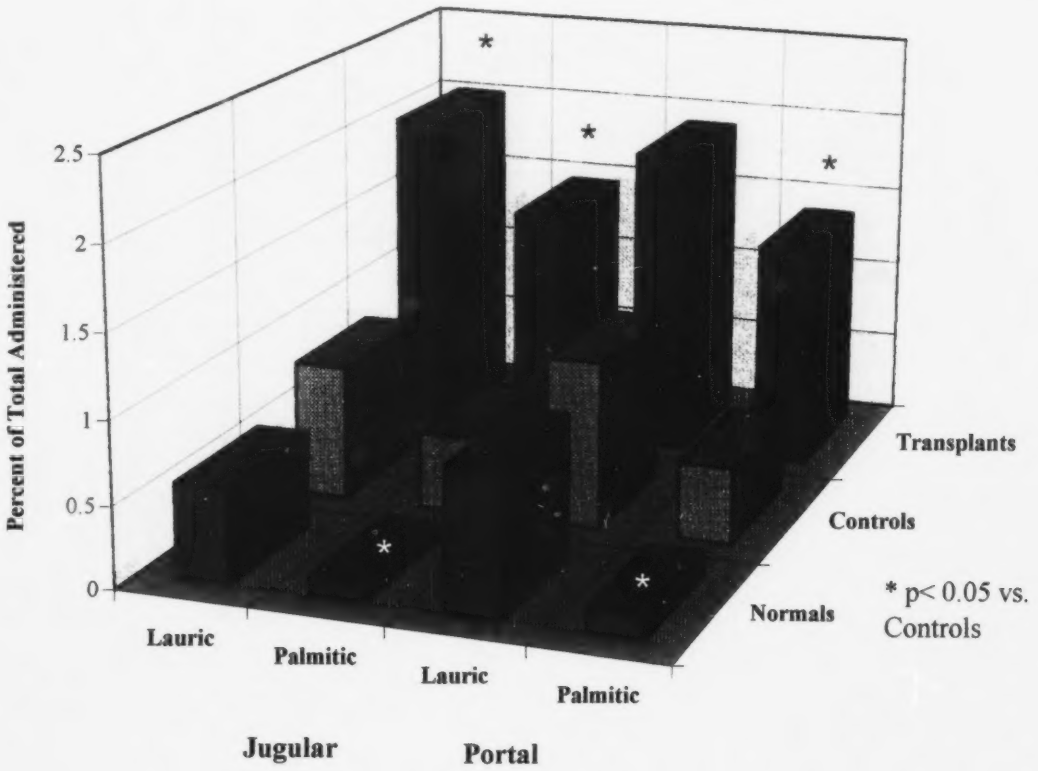
Portal Blood Fatty Acid Recovery

		Percent Fat Recovered	
Group	***n***	**Lauric Acid**	**Palmitic Acid**
Normals	6	0.87 ± 0.20	0.15 ± 0.05*
Controls	12	1.00 ± 0.55	0.43 ± 0.58
Transplants	6	1.9 ± 1.1*	1.38 ± 0.86*

Means ± S.D.

* $p < 0.05$ *t*-test vs.Controls

Figure IV-6
Mean Blood Absorption of Fatty Acids



Chapter V - Discussion of Results

As described initially, fat absorption is a complex process entailing multiple steps both in the lumen of the bowel as well as in the enteric mucosa[160,182,183]. It is clear that the lymphatics play a major role in the absorption of fat[13,76]. Although traditional teaching asserts that this is only applies for long chain fatty acids, the fact remains that lymphatic disruption will affect the intestinal ability to absorb fat and transport it to the systemic circulation for use by the organism. Furthermore, if traditional teaching is true, the only essential fatty acids, linoleic and linolenic acids, are both unsaturated long-chain fatty acids and their absorption would surely be affected by manipulation of the lymphatics. This is an important facet in the development of clinical small bowel transplantation since the ultimate goal of this procedure is to allow the graft recipient to receive all their nutritional requirements, fat included, from enteral sources alone.

To reiterate, the literature has demonstrated lymphatic regeneration following SBT since 1966[87] and many studies have demonstrated normalization of fat absorption following SBT.[59,145,163,170,177] However, an evaluation of the function of these regenerative lymphatics and their role in re-establishing fat absorptive function, although implied in the literature, has not been directly performed. As outlined in the introduction, this direct observation was one of the aims of our study.

The results of this study demonstrate the lure of experimental science: how it is possible to set out to find answers to one set of questions, and while answering them, discover something new or different which prompts the asking of another set of

questions. Thus it is that this discussion has three foci. The first of these is a discussion of the physiologic processes which occur in normal animals, thus providing a basis for our subsequent studies. The second is an analysis of our results as they pertain to the fat absorption process following small bowel transplantation - the main thrust of this study at the outset. Finally, we will discuss our findings with regard to the absorptive routes of medium-chain fatty acids.

Fat Absorption in Normal and Control Animals

In order to substantiate our investigative model, we started by analyzing the temporal relationships of fat absorption in a group of normal animals. In these studies we found that the primary route of fat absorption is indeed the mesenteric duct. It is via this route that 51% of administered lauric acid and 59 % of palmitic acid are absorbed by twenty hours. More important to our study, graphical analysis of the time sequence of this absorption shows that large amounts of the radio-labelled fat are absorbed early on, peaking at 60 minutes, falling quite precipitously until 120 minutes and then gradually tapering to the end of the twenty hour sampling period. Thus, we showed that the most significant amount of fat absorption, 63% of the lauric acid and 61% of the palmitic acid, was absorbed in the first four hours.(Figure V-1)

Long-term studies in control animals parallelled the time course of fat absorption via the mesenteric duct demonstrated in normal animals. That is, peak absorption occurred at 45 minutes and fell rapidly again to 120 minutes. By four hours, 77% of the total absorbed lauric acid and 76% of the total absorbed palmitic acid were recovered

from the mesenteric duct of control animals. Furthermore, a secondary absorptive peak for both fatty acids at 150 minutes was also reproduced in the control model.(Figure V-2)

As the immobilization is known to be stressful for the animals, this information allowed us to justify our sampling over four hours in order to minimize the stress to the animals in the test groups, yet provide a comparable period of study indicative of the true nature of the physiologic events surrounding fat absorption.

The jugular and portal venous studies in normal animals also demonstrated that very small amounts of the radio-labelled fat appear in these circulatory beds. Thus, any increase in the appearance of radio-label in either venous system would indicate an alteration in fatty acid absorption physiology.

The results of the cadaveric studies in the normal group allow us to attempt an accounting of the fate of absorbed fat. However, they also demonstrate that accounting for 100% of the administered fat is very difficult. If we add all the recovered fat at twenty hours from the available sites (the mesenteric duct, the blood, the liver, bowel and carcass), the total measured lauric and palmitic acid is 62% and 67% of the total administered respectively. Thus, we are left with an unaccounted deficit of 35% of the initially administered fat.

The factors involved in the creation of this deficit are likely multiple. Firstly, using a scintillation chamber for the counting of radioactive decay events imparts a systematic error into the study due to "quenching". In order for a scintillation counter to work, the radioactive substance must be placed in a "scintillation cocktail" which fluoresces with each radio-decay event. The light given off by the cocktail is picked up

by a photometric device in the chamber which then tallies the event, thus counting the decay events. However, pigments such as hemoglobin in the blood samples, may absorb the light given off by the cocktail, thus not allowing it to register with the photometric device. This absorption is referred to as quenching and results in an underestimation of the amount of radiolabel present in the sample. One way of reducing this error is to perform fat extractions on all the samples, thereby reducing the pigment content and therefore the quenching in the samples. However, this is a very laborious procedure given the number and very small volumes of the samples taken.

The amount of each fatty acid recovered from the lymphatics at 20 hours following administration of correlates well with the work of Tso et al.[191] In their studies, they showed that with lower hourly doses of FA administered, lower recovery of that FA in lymph occurred. Conversely, with higher doses of FA, greater amounts of FA are recovered in the lymph. The use of 40 μmol of each FA in our model and our subsequent recovery of 51% and 59% of the total administered lauric and palmitic acids respectively fits well with their dose-recovery data. This would indicate that our administration and mesenteric duct sampling techniques are not in error and probably do not contribute to underestimating FA recovery from the animal.

In the carcass studies, several technical problems may have contributed to an underestimation of the fat recovered. Firstly, from our balance studies, it would be reasonable to expect that if about 80% of the fat is absorbed, we should find about 20% in the bowel. Our residual fat in the bowel was less than 10 percent for both FA. Part of this may be due to loss of luminal fluid through reflux into the stomach and loss into the

peritoneal cavity during the harvesting of the bowel. Clamping of the bowel ends prior to mobilization and careful excision technique may help to improve the recovery of the fat from the bowel. Secondly, the preparation of the carcass itself is a difficult undertaking. The carcass must be autoclaved in water at 100 degrees Celsius in order to soften the tissues and bones for homogenation. During this time, FA is released into the water and forms an effluent on the surface which is very difficult to recover. Furthermore, the temperature is sufficient to cause volatilization of both of our radio-labelled FA. Consequently, our extremely low recovery of both FA from the carcasses of these animals probably substantially underestimates the true situation.

Finally, there may loss of the labelled fat through the accessory mesenteric duct. This duct lies inferior to the superior mesenteric artery and, although half the size of the main duct, is obvious under the operating microscope. Due to its size, it is extremely difficult to cannulate. However, despite achieving a stable lymphatic flow through the mesenteric duct cannula, one wonders if this still produces relative duct obstruction. If so, there may be a shunting of fat-laden lymph, with its label, into the accessory duct and subsequently into the systemic blood via the cisternae chyli/thoracic duct system. The degree of shunting might be measurable in one of two ways. The first is through cannulation of the accessory duct itself, although this presents an extreme technical challenge. The second method is by cannulating the thoracic duct, although this also is technically challenging and samples would be tainted by the lymphatic return of the lower body. Thus, it is possible that this system contributes to the deficit identified in our normal animals, however, our ability to quantify it is limited.

The final consideration in trying to account for all the labelled FA administered is that the eventual fate of ingested fat has never been delineated before. Essentially, the assumption has been made that any difference in the amount of fat recovered in the lymph and stool compared to the total administered was due to portal absorption of the FA. However, in our direct studies of portal absorption, we showed that this is not the case. It appears, then, that further work in regard to normal fat absorption and the fate of ingested fat is warranted to more fully understand this complex physiologic process. Unfortunately, several technical barriers will have to be overcome before this is possible.

Fat Absorption Following Small Bowel Transplantation

The results of our balance studies indicate that dietary fat absorption is maintained at six weeks following SBT. This agrees with the findings of other reported metabolic studies.[156,163,170,177] However, the 10% diet fed to these animals is considered to be low in fat. In human clinical circumstances, the normal North American dietary fat intake of 35% far exceeds the level used in our studies and consists of a mixture of FA, most being saturated LCFA. The diet the rats were fed in these studies consisted mostly of saturated and polyunsaturated LCFA. Thus, our choice of diet for these balance studies, and therfore their results, may not be completely transferable to the clinical situation. In addition, the transplanted bowel may have a threshold above which it cannot maintain normal fat absorptive function. This area needs further study and has

obvious clinical ramifications in that steatorrhea may occur unless lower fat dietary adjustments are made following SBT.

The route by which this fat is absorbed is significantly altered according to the results of our study. The mesenteric duct, the primary route of lymphatic drainage, has a significantly reduced role in fat absorption following SBT. We found that the average absorption over the four hours was approximately one-fifth that of the control animals for both fatty acids. However, the flow through this duct was also very significantly reduced in transplant animals, also being about one-fifth that of the control animals. Our calculation of fat transport via this route is very obviously dependent upon the rate of flow through the duct. Thus, if flow through the mesenteric duct is reduced, the capacity to transport fat by this route is also reduced.

That flow of any substantial amount occurs through this duct following SBT is an interesting finding considering the anatomic disruption induced by the procedure. The operation disrupts the mesenteric lymphatics as they begin to coalesce in the base of the mesentery. The condensation of these lymphatic channels into the mesenteric duct is not taken with the graft, but it is left behind when the recipient bowel is removed. However, when the graft is placed in the recipients's peritoneal cavity, the vascular anastomoses are performed at least one centimetre inferior to the original position of the bowel. Thus, the raw edge of the graft mesentery is also not aligned in its original position. With time, however, it would appear that at least some of the mesenteric lymphatics are able to form connections with the native mesenteric duct despite its distance. Kocandrle et al. demonstrated radiographically that the mesenteric lymphatics will re-cannulate to the

mesenteric duct in canine auto-transplants with careful re-approximation of the severed mesentery to its bed.[87] Thus, it is reasonable to expect this behavior following an isogeneic SBT.

Another origin of the lymph passing through the mesenteric duct post-SBT may be the retained recipient duodenum. This part of the small bowel is not disturbed and does not have its lymphatic connections to the mesenteric duct severed. Therefore, its contribution to the mesenteric duct flow should not be compromised by the transplant procedure

It is clear, however, that despite the presence of the retained duodenum and the likelyhood of formation of mesenteric lymphatic connections to the mesenteric duct, return of normal flow through the duct does not occur. Two explanations for this exist: the size of the regenerated lymphatics, and the number of lymphatic channels rejoining the mesenteric duct.

Very often in biological systems, regeneration of tubular structures such as blood vessels results in thin, tortuous vessels, as noted in wound healing or retinal neovascularization in diabetes. The same probably occurs with these lymphatic connections. Furthermore, they are substantially longer than in the normal situation, having to traverse at least one centimetre of retroperitoneum in order to reach the mesenteric duct. These connections then follow Poiseuille's law:

$$\text{Flow} \propto \frac{R^4}{\ell}$$

Thus, the small radius of these channels has an extreme effect upon reducing the lymph flow through them. The increased length required to make the connection to the duct

also negatively affects flow, but does not have the same degree of impact as the radius does. An extrapolation of this law is that a small radius will also cause an increased resistance in the vessel. Thus, if the flow-inducing pressure remains the same in the mesenteric lymphatic bed following transplantation, these small-calibre lymph channels should ostensibly prolong the time required for lymph to drain into the mesenteric duct.

In addition, since the distance from the graft mesentery to the duct is relatively long, it is not unreasonable to predict that only the most cephalad lymphatics of the graft mesentery will successfully unite with the native mesenteric duct. Thus, the absolute numbers of mesenteric lymphatics emptying into the duct may also be substantially reduced, thereby reducing the volume of lymph emptying into the duct even further.

With these factors playing a role, then, re-establishing a flow of even one-fifth normal through the mesenteric duct is testament to the regenerative abilities of the lymphatic system. However, observation of the time sequence of fat absorption through the duct demonstrates an almost identical pattern as that seen in control animals. That is, a peak in maximal absorption at 30 minutes, and a subsequent precipitous drop to 120 minutes, where the absorption plateaus except for a slight rise at 150 minutes.(Figures V-3 and V-4)

Although the height of the curve is significantly reduced along its length when compared to controls, the shape of the curve and its mirroring of the normal physiologic situation yields some interesting insight into the function of the enterocytes and lymphatics and their role in fat absorption following SBT. Since the temporal events marking the absorption of fat via the lymphatics, ie. the absorption peak, remain intact, it

would appear that luminal absorption and intra-cellular packaging of fat are unaffected by the transplantation process itself. Whereas the de-nervation of the bowel has been shown to alter mucosal function with respect to chloride and water secretion[202], it probably has no effect on the passive function of fat absorption. Thus, it appears that the fat absorptive function of the enterocyte is not impaired by the transplantation process. The transfer of the chylomicrons into the lymph and their flow into the mesenteric duct are also not affected according to the temporal maintenance of the absorptive peak in SBT animals. Thus, if flow in this situation was in fact reduced due to Poiseuille's law alone, the small diameter regenerative lymphatics would experience a high resistance to flow. Thus, it would take a substantially longer period of time for the absorptive peak to appear in the mesenteric duct in transplanted animals than in controls. However, we can see that this is not the case. If anything, the peak appears somewhat sooner in the transplanted animals. Therefore, the effect of Poiseuille's law in forming lymphatic connections to the mesenteric duct does not appear to have the impact one might expect. This is borne out by morphologic studies by Schier et al. who demonstrated that although initial lymphatic regenerative channels are small, they gradually dilate with time.[148] Thus, as their radius increases, Poiseulle's law becomes less important in determining the flow capacity of the regenerative networks.

On the other hand, the uniform reduction in the height of the curve indicates that the volume of lymph delivered to the duct is primarily responsible for the reduction in fat absorption via this route. Since the time course of absorption remains intact, increased resistance to flow can be ruled out. We are therefore left to deduce that reduction in

volume must be due primarily to reduced numbers of lymphatics actually making the regenerative connection with the native mesenteric duct.

With the objective evidence demonstrating a loss of function by the mesenteric duct, we are still left with the fact that overall dietary fat absorption is maintained. Thus, the bowel must be able to adapt in some way in order to make up for the decrease in absorption via the mesenteric duct. Again, two possibilites exist: the bowel adapts its function by increasing the portal venous contribution to absorption, or, mesenteric lymphatics which do not re-join with the mesenteric duct form new connections in order to drain fat-containing lymph from the bowel.

In order to evaluate these possibilites, we focus on the results of our jugular and portal venous studies. The amount of both fatty acids appearing in the jugular venous blood is significantly higher in transplanted animals than in control animals. This is also the case in the portal blood of transplanted animals. When we look closely at the relative values of fat appearing in the two venous systems, we note that the jugular quantity is higher than the portal. If the portal system were contributing in any substantial way to the absorption of fat from the bowel, one would expect that the amount of fat recovered from the portal blood over the sampling period would be substantially higher than that in the jugular blood. The relatively small volume of blood in the portal circulation into which the fat is absorbed should yield a higher concentration of fat recovered in the portal blood. Furthermore, mixing of the portal and systemic circulations would result in distributing the portal-derived fat throughout the much larger systemic vascular volume of the animal. In our model, this mixing would occur rapidly, given the porto-systemic

shunt we create following SBT thus enhancing the absorbed fat's dispersion in the systemic circulation and creating a more pronounced drop in the systemic blood level when compared to the portal blood level. Hence, fat recovery from the systemic vasculature, via the internal jugular vein, should be lower than that of the portal blood if the portal system is substantially contributing to enteric fat absorption. This is obviously not the case in our study which shows a minimally higher jugular fat recovery than portal recovery. Therefore, it would appear that an adaptation by the enteric vasculature to increase the absorption of fat by the portal system does not occur. Instead, the higher recovery of fat in the portal vein of transplants versus controls is most probably due to re-circulation of the elevated jugular fat levels.

If increased portal absorption is not contributing to the appearance of significantly higher levels of fat in the jugular vein, another route must exist through which the bowel is able to absorb fat and transport it into the systemic circulation. The only remaining explanation lies with the fate of those mesenteric lymphatics which do not re-unite with the mesenteric duct - a significant number if our theories regarding mesenteric duct flow are true.

To more closely evaluate these lymphatics, we utilized the injection of methylene blue into mesenteric lymph nodes in selected transplanted rats. As outlined in our methods, we chose a lymph node in the periphery of the graft mesentery and injected it with 0.5 mL methylene blue dye. We then observed the egress of the dye from the mesenteric lymphatic system, timing its appearance in identifiable lymphatic beds.

Within 15 minutes of injection, dense staining around the arterial anastomosis was noted. Thus, it appears that a significant regenerative lymphatic bed develops around the superior mesenteric artery. (Plate IV-1) Furthermore, a significant lymphatic vessel associated with the left lumbar vein in the retroperitoneum was also noted to be carrying methylene blue. This finding in itself is interesting because under normal circumstances, retroperitoneal lymphatics have no role in draining the lymph from the bowel. However, following transplantation, we find that retroperitoneal lymphatics are now conveying lymph from the bowel. This must mean that, along with regeneration around the anastomosis, further regenerative networks must also form in the retroperitoneum.

Following the movement of the dye from these networks, we observed that they drain into a large para-caval lymphatic running along the left side of the inferior vena cava. (Plate IV-2) From here, the lymphatic empties into the cisterna chyli and then into the thoracic duct and subsequently into the systemic circulation. Thus, these lymphatics transport fat-laden lymph into the systemic circulation and this accounting for the higher recovery of fatty acid in the systemic blood.

We further noted the transport of dye into the mesenteric duct starting at 30 minutes from injection. This correlates well with the appearance of the absorptive peak in the mesenteric duct demonstrated previously in transplanted animals. We can only speculate about what physiologic indication this gives us. However, it may demonstrate that lymphatic flow is relatively constant and that the bowel absorbs fat most rapidly in the duodenum and upper jejunum, thus producing a peak in absorption. If so, then this

also helps to validate our steady-state lymphatic flow model in these experiments in addition to Tso's experiments.[189] The mesenteric duct then flows into the same large paracaval lymphatic draining the perianastomotic and retroperitoneal lymphatic beds.

The para-caval lymph channel is not readily demonstrated in normal or control animals. Presumably this is because there is relatively less lymph flowing through it in these animals. It probably is part of the para-aortic lymphatic chain.[148] However, once the perianastomotic and retroperitoneal beds join with it, it becomes much more distended simply because of the amount of lymph it must drain from the bowel. In fact, the diameter of this lymphatic is about the same as the normal mesenteric duct, about 1 mm, indicating an enormous increase over the normal situation in the volume of lymph it is transporting due to these new connections. Furthermore, the wash-out period of the dye in the regenerative beds is relatively short - approximately 30 minutes. This would indicate that the flow through these networks is relatively brisk. Thus it would appear that these lymphatics are able to transport fat-laden lymph in sufficient quantities to make up for the deficit demonstrated by the mesenteric duct.

The presence of these regenerative lymphatics have been documented in past studies,[87,192] using methylene blue and contrast media injected into mesenteric lymph nodes of grafted small bowel. Schier did detailed studies using microlymphangiography in identifying regenerative lymphatic anastomoses.[148] He found that these beds formed in three stages. Initially, the lymph in the mesentery does not flow at all. Once the fourth post-operative day is reached, however, the lymph finds its way into "pre-existing adventitial lymphatic vessels" along the graft mesenteric artery but stops at the level of

the arterial anastomosis. The lymph then crosses the anastomotic line by the sixth post-operative day and flows along the aorta of the recipient. By the eighth postoperative day, he observed aortic adventitial lymphatic connections with other, established, lymphatic beds. One of these beds was the retroperitoneal vasa lymphatica testicularia sinistra paralleling the left testicular vessels. In addition, his group identified a substantial ascending lymphatic along the aorto-caval bundle similar to that found in our methylene blue studies.

Tsuchiya et al.[192] also noted regenerative lymphatic beds in a model utilizing a porto-portal anastomosis for the graft. Their demonstrations were not as detailed with regard to the regions in which these beds formed. However, they did show that regeneration took a little longer in this model than in Shier's porto-caval model. They suggested that this difference may be due to operative methods or morphologic differences between the lymphatics of the differect rat strains used. However, Shier's demonstration that the arterial anastomosis serves as the primary site of at least one of these regenerative networks would indicate that it is the site of implantation of the graft SMA into the recipient aorta which is most important and not the method of venous drainage.

Another possible fate of the severed mesenteric lymphatics is that they empty ito simple spaces in the retroperitoneum. This regenerative possibility arises from studies surrounding the re-vascularization of cellular grafts such as pancreatic islets.[41] It appears that capillary networks never really form around these grafts. Instead, the arterial capillaries simply dump into a space surrounding the graft, bathing the cell in

blood. The venous capillary then removes the blood at another point in the "sinus". Thus, it may be that some of the mesenteric ducts never re-cannulate, but instead empty into lymphatic "lakes" in the adipose of the retroperitoneum. It is well-known that adipose tissue contains lipoprotein lipase capable of removing TG from CM and VLDL.[187] Thus, once these lymphatics transfer their load of lymph into these "lakes", the adipose tissues surrounding them may be able to absorb the transported TG, thus contributing to fat absorptive function via a mechanism not measured by our model. Neither Tsuchiya or Schier were able to demonstrate the development of "lymphatic lakes", but it is possible that this occurs on a microscopic level not visible using their techniques. However, with the degree of lymphatic re-cannalization demonstrated throughout the literature, it is likely that the development of lymphatic lakes in this situation contributes to fat absorption in a very small way, if they form at all.

Our methylene blue studies, although cruder than those performed by Schier, confirm that regenerative lymphatic beds form in much the same way he describes. That is, it appears that perianastomotic and retroperitoneal regenerative beds develop in order to drain the portion of the bowel no longer serviced by the mesenteric duct.

However, the fact remains that no direct studies of the function of these beds have been performed to date. The literature contains assumptions correlating the regeneration of these regenerative lymphatic beds with normal fat absorption, but no quantitative data has been asserted.[55,152,153]

Our studies confirm the observational studies above. However, the demonstration of significantly higher jugular fat recovery following transplantation indicates that an

alternate, non-vascular route of fat absorption is being utilized by the graft. Thus, it would appear that these regenerative beds around the arterial anastomosis and in the retroperitoneum, although proven to be patent by numerous studies, are also functioning in the fat absorptive process. Furthermore, due to the reduced drainage through the mesenteric duct, it also appears that these regenerative beds shoulder the majority of the lymph flow, thereby helping to maintain fat absorption by the transplanted graft.

Altered function of regenerative lymphatics has ben anecdotally noted in the clinical setting of rejection following renal transplantation (personal communication, Dr. G. Todd, Associate Professor, Dept. of Surgery, University of Alberta). It appears that with the onset of rejection, the regenerative beds become blocked and may even burst to form lymphoceles. Presumably, these lymphatic channels become blocked with infiltrating leukocytes, attracted by the rejection response. This altered lymphatic function has also been indirectly noted by Schindler et. al.[149] who demonstrated a fat malabsorption state with the onset of rejection following SBT. Again, this may have been due to obstruction of the regenerative channels with leukocytes, although this was not observed experimentally in his studies.

In summary, it regenerative lymphatic beds develop following SBT. These beds appear to function in the maintenance of fat absorption following SBT as demonstrated directly through the use of the simultaneous portal vein cannulation/lymph fistula rat model. In addition, they may also function in the immunologic response to the graft but more detailed studies regarding this function will be required.

Absorption of Medium-chain Fatty Acids

A very interesting finding in these studies was an apparent equality in the amount of medium and long-chain fatty acids absorbed into both the lymphatic system as well as the portal circulation. For both transplants and controls, we were unable to identify significant differences in absorption based on chain length.

Obviously, this finding is in stark contrast to the traditional teaching based on the work of Bloom and Chaikoff in 1951.[13] The studies they performed at that time laid the foundation for the study of very fundamental physiologic processes in fat absorption. In their results, they demonstrate a preferential absorption via the mesenteric duct of fatty acids having more than sixteen carbon-chain lengths than those having fifteen or less carbon chain lengths. They then assumed that the difference in absorption of these medium chain fatty acids was absorbed via the portal blood, thereby maintaining the overall absorption of these molecules. A measurement of the appearance of the fatty acids in the portal blood was never made.

The differences seen in our study with respect to theirs are numerous and may account for the differences in the results we obtained. Firstly, they had to manufacture all their own fatty acids and attach carbon-14 labels to them. In this situation, then, the purity of the substances used may not have been standardizable. Hence, bioavailability of each administered dose may have also fluctuated, thus gving inconsistent results. Indeed, the range of lauric acid absorption via the mesenteric duct was 15 - 55% with an average of 36.4% over a twenty-four hour period. We were able to isolate an average of 51% of the administered lauric acid in normal animals and 43% in control animals in

twenty hours, suggesting reagent quality may play a role in the outcome of studies such as these. Even so, we were not able to demonstrate significant amounts of this medium chain fatty acid in our portal samples. Secondly, they did not ensure a steady state lymphatic flow which has been shown to have a major impact on studies such as these[155], thus potentially confounding the results of their study as well.

Isselbacher published work in 1968 confirming the preferential absorption of medium chain fatty acids via the portal vein by direct measurement of portal blood, although their methods for this were not completely described.[76] It appears that these portal samples were acquired one hour after instillation of radio-labelled MCFA and that sacrifice of the animal was required to obtin the sample. A continuous physiologic model did not appear to be used. Instead, a "snap-shot" of absorption was created. Furthermore, it is very important to note that these confirmatory experiments utilized fatty acids in the short range of the medium-chain spectrum. Specifically, C8:0 and C10:0 fatty acids were used.

In contrast, our studies showed that there was no difference in the absorption of a mid-range medium chain fatty acid into the lymph or portal circulation when compared with a long-chain fatty acid. This pattern was demonstrated in all three groups of animals, suggesting that despite the physical effects of small bowel transplantation, the basic cellular processes for absorbing fat are maintained.

In addition, the temporal appearance of both fatty acids in the lymph was also the same. In fact, the absorption rate curve was almost exactly the same for both fatty acids. A delay in the appearance of fatty acids in the lymph has been noted in the

literature.[69,189] The explanation for this delay is that fat being transported via the lymph is either in the form of chylomicrons or very-low density lipoproteins. As such, absorbed fat must be processed by intracellular organelles and the transport particles then constructed, all of which require a finite period of time to be accomplished. Hence, if one accepts the fact that long-chain fatty acids are transported almost solely via these particles in the lymph, as confirmed throughout the literature[69, 188-191], then another fatty acid following the same temporal absorptive pattern must ostensibly also be absorbed and processed in a similar manner. The identical nature of the curves for both fatty acids in all groups helps confirm the significant role played by the lymphatics in absorbing longer medium chain fatty acids in the form of CM and VLDL as well as long-chain fatty acids.

In summary, the similarity of the absorption curves of both lauric and palmitic acids indicates that the MCFA lauric acid is treated like a LCFA by biologic systems. The reason for this is not entirely clear.

When defining a medium-chain fatty acid, we do so using a variety of chemical measures. These include solubility factors, melting and boiling points, and other quantifiable properties which change measurably with chain length. Although the exact cut-off for defining the change from medium to long chain FA is not absolute, the trends seen in the FA physical parameters in Table V-1 demonstrate that with increasing chain length, melting and boiling points rise, specific gravity drops, and refractive index rises. Furthermore, shorter length FA are more soluble in smaller molecular organic solvents than longer chain.

Chemically defined, then, lauric acid (C12:0) is a medium chain fatty acid. It was on this basis that we decided to us it in our study to compare with palmitic acid, a chemically defined long chain fatty acid. However, it appears from our results that lauric acid is incorporated into CM and VLDL particles and transported primarily through the lymph. Again, this is apparent by the similar lymphatic absorption curves for these two FA. Ockner and Manning's work suggests that the first step in processing an absorbed FA for eventual packaging in CM and VLDL is its binding by FABP[120] The properties of the binding site have been elucidated as well and it appears that steric conformational changes are required for proper FA binding.[143] However, these binding studies have been done only with palmitic acid and longer chain FA. Thus, it is possible that lauric acid, although chemically defined as a medium chain fatty acid, may still be able to undergo the conformational changes required for its proper binding by FABP. Once bound by FABP, it would then be channeled into a re-esterification pathway leading to its eventual discharge into the lymph as CM or VLDL.

While the threshold in chain length for FABP affinity has not been experimentally confirmed, our results provide indirect evidence that FABP affinity may not be be limited to chemically-defined LCFA. In this regard, it appears that lauric acid is treated not as a MCFA in its chemical sense, but as a biologic LCFA, undergoing the same intracellular processing as palmitic acid. Furthermore, our results also help, indirectly, to confirm that the differential handling of absorbed fatty acids by the enterocyte does appear to be based on the affinity of a particular FA for FABP[120] and that FABP constitutes an important regulatory gate for intracellular FA metabolism in the enterocyte.

Further studies using x-ray crystallography using a spectrum of short, medium and long chain fatty acids must be done in order to directly establish the chain length threshold for FABP. In addition, this information must be correlated with studies on the appearance of those same fatty acids in the lymph and portal circulation using a model similar to our own. In this way, verification of the regulatory role of FABP in fat absorption and the biologic handling of FA of varying chain lengths may be obtained.

Clinical data to support our experimental discrepancies with established teaching also exist. Chylothorax and chylous ascites are conditions in which lymph, or chyle, collects in the thoracic and peritoneal cavities respectively. It often has a surgical cause in which trauma to the cisterna chyli or thoracic duct leads to ongoing leakage of lymph. Since a major portion of the leaking lymph has its source in the intestine, logic would dictate that reduction of this flow should reduce the lymph load in the ruptured lymphatic, thus improving the chances of healing the injury. As a result, treatment has been based on total bowel rest with total parenteral nutrition until healing occurs.

However, enteric absorption of glucose and amino acids occurs via the portal circulation. Thus, the dietary variable which really needs manipulation in this condition is the fat content. Recently, interest in the use of medium-chain triglyceride dietary therapy for these conditions has grown. The rationale for this therapy is that, if the theory regarding preferential absorption of medium chain fatty acids via the portal vein is true, then these patients could heal while on enteric diets. In addition, they would not be faced with the problems associated with the central venous access required for total parenteral nutrition.

However, the literature surrounding the treatment of these condition with this dietary therapy have demonstrated limited success[44,52,93,97, 128,194]. These studies are often based on case reports and thus, generalizations are difficult to make. However, it is possible in many of these cases, non-surgical resolution of the problem may well be due to time as much as to the dietary approach. Many of the MCT therapies utilized contain significant quantities of C10 - 12 chain length fatty acids as part of the MCT component. Our studies suggest that the C12 fatty acids will still be absorbed via the lymphatics, and therefore, reduction of lymph flow will not occur when these molecules are administered. Indeed, the amount of lipid collected from chylous ascites dropped to their lowest levels when administering MCT therapies with less than 20% C10 -12 fatty acids.[44] In some studies, total lymph accumulation also was reduced with diets containing less C10 - 12 fatty acids.[52] Thus, it would appear that our experimental findings, although contradictory to established physiologic teaching, are borne out by the clinical data.

Until confirmatory studies in the binding properties of FABP are performed, it would seem that our biologic definition of a medium chain fatty acid, may require revision. As stated previously, further studies using our model of simultaneous portal cannulation and lymph fistulization and a spectrum of fatty acids, may be able to more accurately identify the threshold in chain length which correlates with maximal portal absorption and minimal lymphatic absorption. With these results, it may be possible to formulate a more specific dietary therapy for disorders causing impairment of function of the lymphatic system of the bowel.

Figure V-1
Mean 20 Hour Lymphatic Absorption - Normals

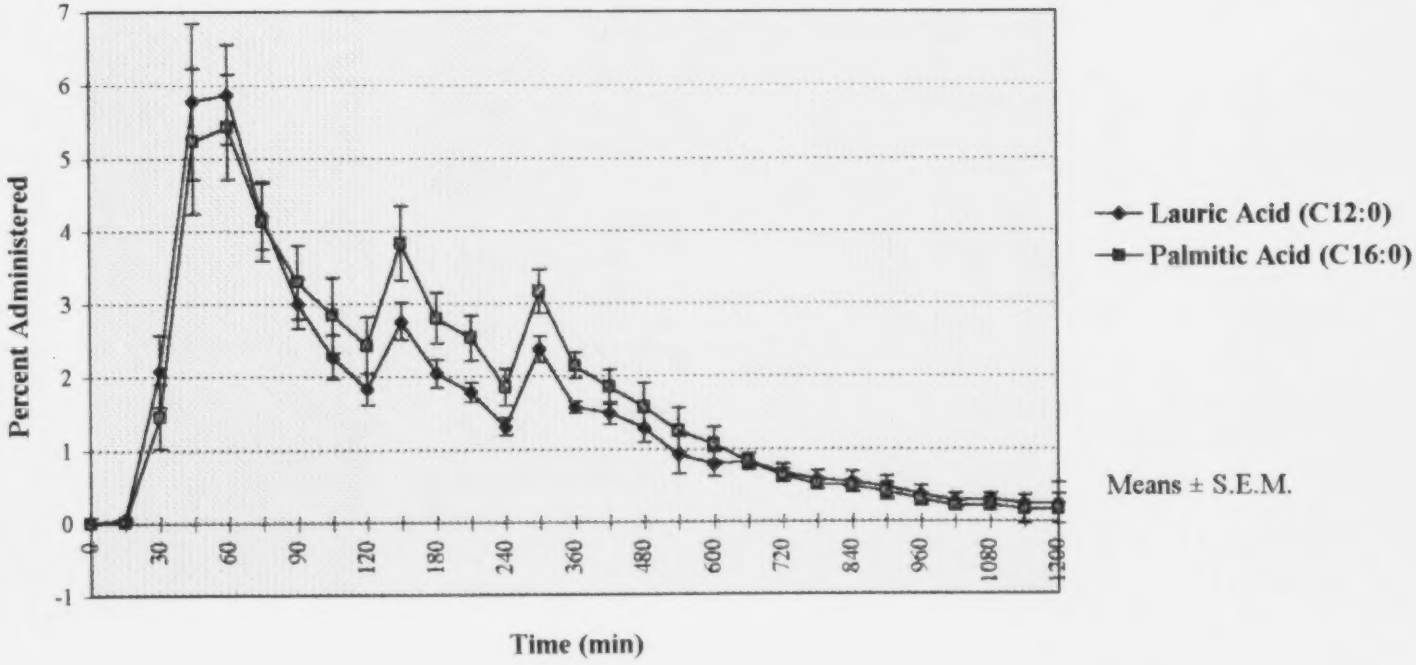


Figure V-2
Mean 20 Hour Lymphatic Absorption - Controls

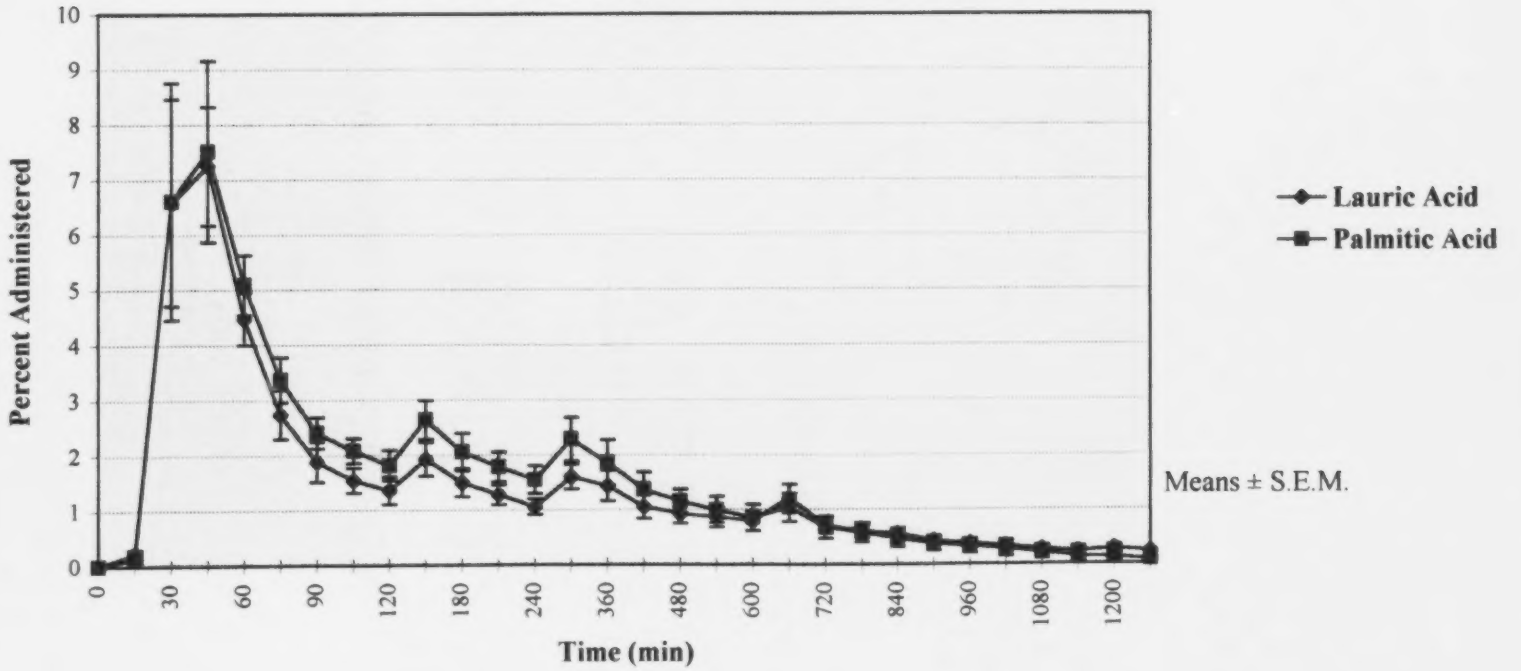


Figure V-3
Mean 4 Hour Mesenteric Duct Absorption - Controls

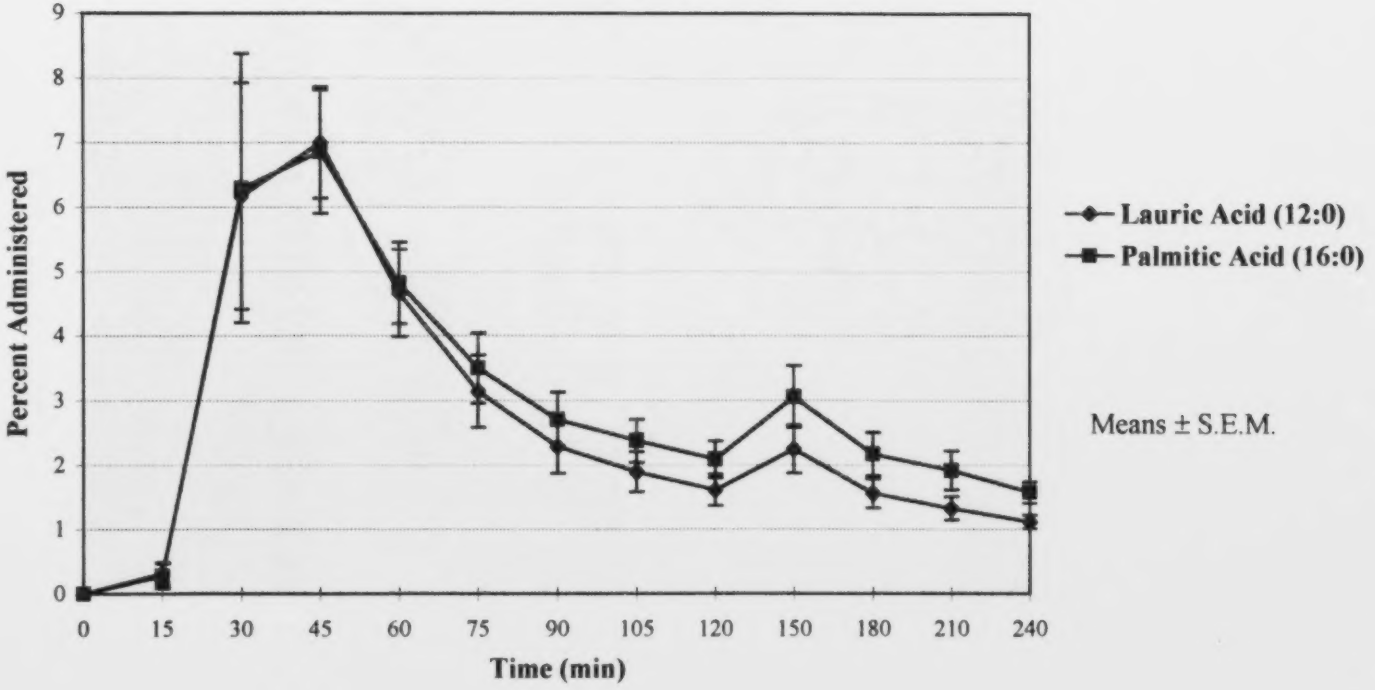


Figure V-4
Mean 4 Hour Mesenteric Duct Absorption - Transplants

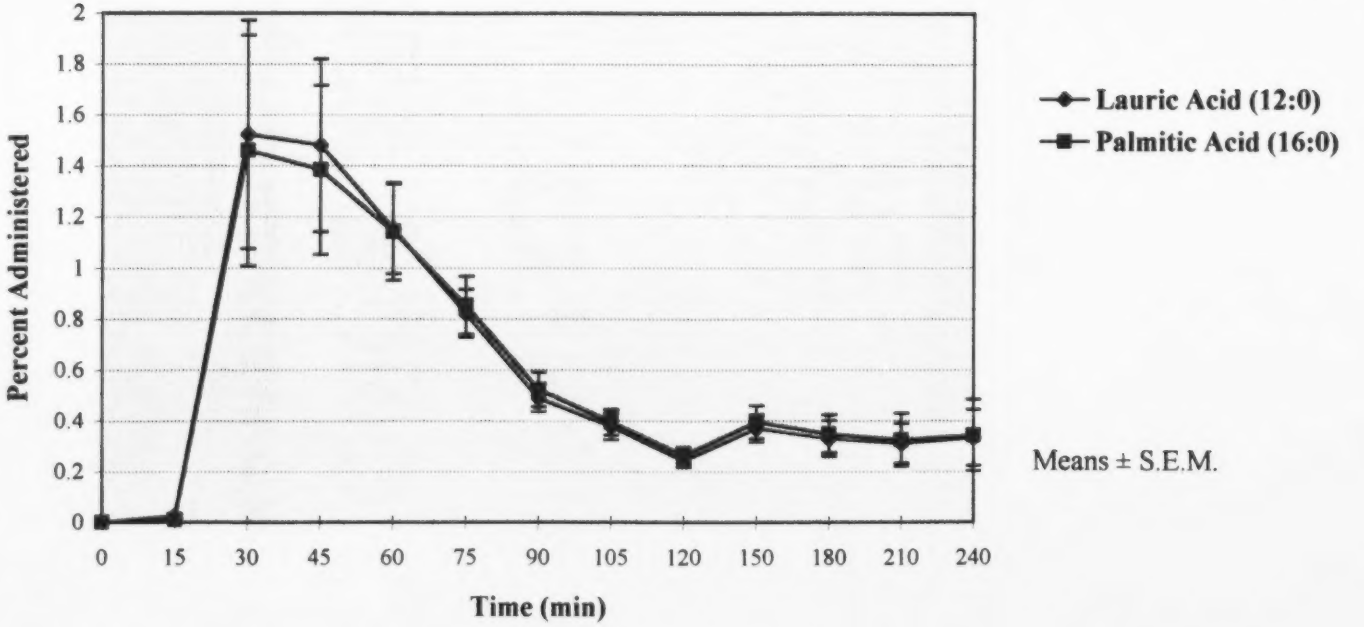


Table V-1

Physical Properties of Selected Fatty Acids of Varying Chain Length[†]

		Lauric Acid (C12:0)		Myristic Acid (C14:0)	
Specific Gravity		d^{4}_{5}	0.869	d^{54}_{4}	0.8622
Boiling Point (°C)		bp_{100}	225	bp_{100}	250.5
Melting Point (°C)			44		58.5
Refractive Index		n^{82}_{D}	1.4183	n^{70}_{D}	1.4273
Solubility:	**Water**		Insol.		Insol.
	EtOH		1 g in 1 mL		Freely
	Propanol		1 g in 2.5 mL		Freely
	Ether		Freely		Freely
	Chloroform		Freely		Freely
	Benzene		Freely		Freely

		Palmitic Acid (C16:0)		Stearic Acid (C18:0)	
Specific Gravity		d^{62}_{4}	0.853	d^{70}_{4}	0.847
Boiling Point (°C)		bp_{15}	215	**bp**	383
Melting Point (°C)			63 - 64		69 -70
Refractive Index		n^{80}_{D}	1.4273	n^{80}_{D}	1.4299
Solubility:	**Water**		Insol.		Insol.
	EtOH		**Cold** - Sparingly **Hot** - Freely		Insol.
	Propanol		Freely		Insol.
	Ether		Freely		Freely
	Chloroform		Freely		Freely
	Benzene		Freely		Freely

Symbols: d^{62}_{4} - density of substance at 62°C referable to water at 4°C
bp_{15} - boiling point at atm. press. of 15 mmHg; 1 atm if not stated
n^{80}_{D} - refractive index of substance at 80°C using sodium light

[†] From Windholz, M, (ed.), Merck Index, 10th edition, 1983. Merck and Co., Inc. Rahway, NJ, USA.

Chapter VI - Conclusions

When this thesis was introduced, several questions were outlined; questions that we hoped to answer by undertaking the studies described and discussed in previous chapters. To reiterate, we wondered about the fate of the lymphatics draining the bowel, both their regeneration and their function. Furthermore, we questioned whether fat absorption was severely altered by the process of SBT, and whether the bowel was capable of re-establishing its absorptive abilities, possibly by increasing portal absorption of fat. The hypothesis we established was that fat absorption following SBT would be decreased following SBT.

Through these studies, we have tested our hypothesis and fulfilled the aims set for ourselves, namely the direct quatification of the fat absorbed through the lymphatic and blood pathways. We have also arrived at answers to our questions. We are able to conclude that our hypothesis was incorrect: dietary fat absorption in a low-fat diet is maintained following SBT. However, it appears that the primary route by which fat is absorbed, the mesenteric duct, has a significant reduction in function after SBT is performed. Instead, functional lymphatic beds developing in the retroperitoneum and around the arterial anastomosis, as demonstrated by our methylene blue studies, allow for the normalization of fat absorption. The evidence for this function lies in our demonstration of a higher jugular fat level, indicating a bypass of the mesenteric duct in fat absorption.

Thus, it appears that patients undergoing SBT may be able to receive their dietary fat requirements by enteric sources only, thereby accomplishing a major goal of intestinal transplantation.

In addition, we were unable to demonstrate a significant difference in the absorption of certain medium chain fatty acids in the lymphatics and portal vein when compared to long chain fatty acids. This finding may prompt further investigation into the physiologic routes of fat absorption based on chain length.

The results of these studies may aid in dietary decision-making, both in patients undergoing small bowel transplantation as well as those with lymphatic collection disorders.

Chapter VII -Future Considerations

The goal of experimental science is to take the work of others and expand on it with the intent of answering new questions. In our studies, we built on the experimental model of Tso and others to develop a simultaneous portal-vein cannulated/lymph fistulized conscious rat. With our results, we were able to establish its validity by comparison with the literature. However, even though we were able to answer the questions we set for ourselves, we are still left with further questions created by our results. There are numerous areas into which the use of this model may be expanded in order to further enhance our knowledge of fat absorption.

First, as mentioned earlier, this model may help us identify the eventual fate of all the ingested fat. However, several technical problems will need to be overcome in order for this to happen. These include cannulation of the accessory mesenteric duct in an adequate fashion and careful harvesting and preparation of the bowel and carcass prior to homogenation. An ability to account for all the fat administered to an animal would only serve to enhance the validity of this model.

Secondly, the area of fat absorption following SBT has barely been investigated, both in the literature and in our own studies. This area of research holds many tremendous opportunities for discovery and the use of our model may aid in that discovery. One problem requiring investigation is a potential threshold in the amount of dietary fat which the bowel transplant is able to absorb. Although we have shown that a network of regenerative, functional lymphatics form to maintain fat aborption post-SBT, these networks may have a limit to their ability to transfer fat-laden lymph. This limit

may be due to the size of the regenerative lymphatics. We know that fat feeding increases CM size[51], thus, with a particular amount of fat administered, the lymphatics may become engorged wth distended chylomicrons. This probelem would be easily addressed using sequential increases in dietary fat administered to SBT rats and performing stool fat extractions to identify the dietary fat level which induced an increase in fat malabsorption. These malabsorption rats could then be sacrificed and their regenerative lymphatics examined histologically to determine if they are responsible for altered fat absorption with increased dietary fat. This study would also be important in determining if fat intake would require strict control in the human SBT situation.

Our cannulation model could also be utilized to extend our current studies and investigate the absorption of poly-unsaturated fats post-SBT. As stated earlier, linoleic and linolenic acids, both poly-unsaturated FA, are essential fatty acids and must either be absorbed enterally or be administered parenterally. A study of this nature would provide clear experimental confirmation of the transplanted bowel's ability to absorb these vital nutrients.

Further to the investigation of fat absorption post-SBT, the use of lauric acid in our studies prevented our being able to determine the function of the portal system following SBT, due to its unforeseen lack of absorption via this route. Thus, it would be interesting to repeat our studies using a shorter chain MCFA such as octanoic or possibly decanoic acid and observe their fate in the portal blood following bowel transplantation. This would allow a more full assessment of the adaptive ability of the portal system, if in fact any adaptation occurs.

Finally, our studies contradicted the basic physiologic literature in demonstrating that, although chemically a MCFA, lauric acid is treated biologically like a LCFA. Also mentioned previously, this may provide indirect confirmation that the regulatory gate in intracellular FA metabolism lies with FABP. Thus, it would be reasonable to undertake a careful investigation using our model and a spectrum of FA from C8:0 to C14:0. Looking at the appearance of these various FA in the portal blood and the lymph of normal animals would allow us to more precisely define a threshold chain length at which the enterocyte changes its handling of FA and subsequent discharge into the blood or lymph. Through this study, we would essentially arrive at biologic or functional definitions of medium and long chain fatty acids which we have already shown to be different than their chemical definitions.

Correlation of these results with those looking at different FA binding affinities to FABP would serve to establish the regulatory role possibly played by this carrier protein, further enhancing our knowledge of the intracellular workings of the enterocyte.

Even if the studies outlined above are performed, they will only prompt the asking of further questions and the development of further investigations. However, this is the means by which our knowledge of the complicated function of fat absorption will continue to expand and become more precisely defined.

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